An Investigation of Microbial Communities Across Two Extreme Geothermal Gradients on Mt. Erebus, Victoria Land, Antarctica

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Abstract

The geothermal fumaroles present on Mt. Erebus, Antarctica, are home to numerous unique and possibly endemic bacteria. The isolated nature of Mt. Erebus provides an opportunity to closely examine how geothermal physico-chemistry drives microbial community composition and structure. This study aimed at determining the effect of physico-chemical drivers on microbial community composition and structure along extreme thermal and geochemical gradients at two sites on Mt. Erebus: Tramway Ridge and Western Crater. Microbial community structure and physico-chemical soil characteristics were assessed via metabarcoding (16S rRNA) and geochemistry (temperature, pH, total carbon (TC), total nitrogen (TN) and ICP-MS elemental analysis along a thermal gradient 10 °C–64 °C), which also defined a geochemical gradient. Diversity increased in alkaline soils suggesting pH to be the primary driver of microbial community structure across the gradients. Archaea dominated the microbial communities at Tramway Ridge compared to Western Crater, which was dominated by Bacteria. Western Crater has been sampled for the first time, widening the understanding of geothermal sites on Mt. Erebus. Organisms that were once considered cosmopolitan may not be as cosmopolitan as expected in Antarctica. These organisms may require specific niches that allow them to colonise new habitats. This study also showed that gathering more samples from Tramway Ridge has widened understanding of the changing environments that microbial communities on Mt. Erebus live in. The study provides a foundation on which to compare microbial and geochemical interactions across all geothermal sites within Victoria Land. This supports the effect of specific geothermal characteristics of the fumaroles in manipulating the microbial communities present.

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List of Abbreviations

ASPA	Antarctic Specially Protected Area
ASV	Amplicon Sequence Variant
BLAST	Basic Local Alignment Search Tool
BME	β-Mercapto Ethanol
Bp	Base pair
CTAB	hexadeCylTrimethylAmmoniumBromide
DADA.	Divisive Amplicon Denoising Algorithm
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
Grad	Gradient
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
NCBI	National Center for Biotechnology Information
PCR	Polymerase Chain Reaction
SCAR	Scientific Committee of Antarctic Research
SDS	Sodium Dodecyl Sulphate
TR1	Tramway Ridge Fumarole 1
TR2	Tramway Ridge Fumarole 2
TRIS	Tris(hydroxymethyl)aminomethane
WC	Western Crater

Chapter 1

Literature Review

1.1 Geothermal Systems

1.1.1 Tectonic activity

Geothermal activity is caused by the movement of multiple tectonic plates that cover the earth's surface (lithosphere) (Trujillo & Thurman, 2008). The movement of these plates is caused by changes in the upper mantle (asthenosphere) through melting and movement at plate boundaries (Trujillo & Thurman, 2008). According to Tectonic plate theory, these plates shift as the ocean floor spreads, governing the movements of the continents above (Cox & Hart, 2009; Trujillo & Thurman, 2008). For example, large continental movement happened approximately 300 million years ago when the two large continental masses, Gondwana and Laurasia, merged together to form Pangaea then approximately 200 million years ago, Pangaea broke up to form the current positions of the continents, which can move at a speed of 2–12 centimetres per year (Trujillo & Thurman, 2008). Tectonic activity has shaped the continental features of the planet (Trujillo & Thurman, 2008).

Plates move in three different ways: divergent, convergent, and transform (**Figure 1.1-1**). Tectonic plates move because geothermal vents on the ocean floor expel magmatic material to the surface in a process causing the ocean floor to spread (Trujillo & Thurman, 2008). Divergent plate movement occurs when the asthenosphere releases magma and pushes the plates apart at areas of thin-crust such as mid-ocean ridges, thus driving the opposite side of the plate into the neighbouring plate in a convergent manner (Trujillo & Thurman, 2008). Transform movements are the sliding of two plates past each other leading to earthquakes. Collectively and separately, these plate movements create areas of heat and pressure release resulting in the formation of volcanoes and geothermal activity at the surface (Trujillo & Thurman, 2008).

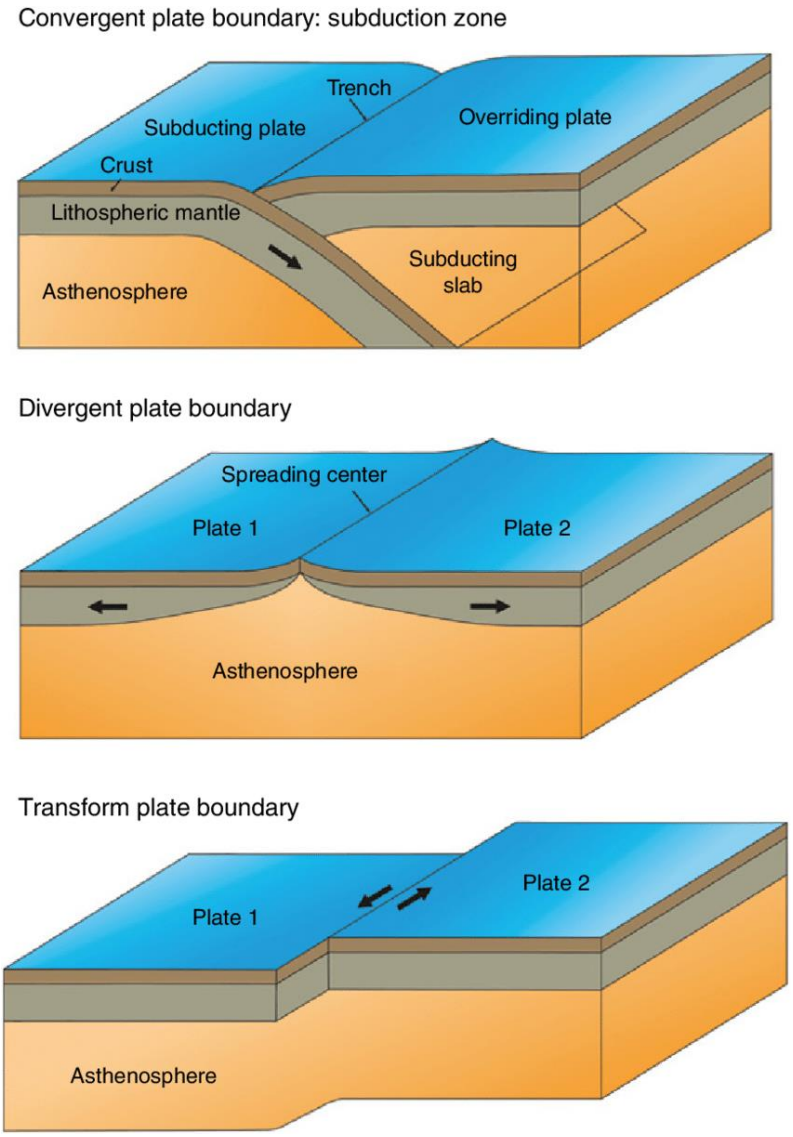

Figure 1.1-1: The types of plate boundaries and movements sourced from (Duarte & Schellart, 2016).

1.1.2 Types of Geothermal Systems

A geothermal system is defined as an area of the earth surface that is heated due to the close proximity of magma (Trujillo & Thurman, 2008). There are several types of geothermal systems, including sedimentary, convective, and volcanic systems (Saemundsson et al., 2009). Sedimentary systems such as the Western Canada Sedimentary Basin occur when tectonic plate movement allows for the earth to cave in and sediments continue to deposit below the erosion level (Saemundsson et al., 2009). These systems exist at plate boundaries, either when the plates collide, pull apart, or slide past each other (Bachu & Burwash, 1991; Weissmann et al., 2015). Convective systems

are geothermal systems that use water to transfer heat to the surface like that of the Salton Sea in California (Procesi et al., 2019). In contrast, volcanic systems are linked to particular volcanoes and are often located on the volcano (Saemundsson et al., 2009). Famous geothermal systems include Yellowstone National Park in the United States, Taupō Volcanic Zone in New Zealand, the volcanoes of Antarctica and Iceland.

There are three types of volcanic systems: rift, subduction, and hot spot. Rift volcanoes occur as the tectonic plates separate from each other, whereas subduction volcanoes form when tectonic plates collide (Trujillo & Thurman, 2008). Hotspot volcanoes are created by intraplate tectonics and are not located on or near plate boundaries (Saemundsson et al., 2009). An example of volcanoes forming from the separation of two plates is the Antarctic rift system which was formed 66 million years ago when the East and West Antarctica microplates separated, leading to crustal thinning and geothermal activity such as volcanoes including Mt. Erebus (Fitzgerald, 2002) These systems occur around the globe and can create unique environmental niches of significance to scientific research (Thomas D Brock, 1967).

Volcanic arcs are volcanic systems that have formed above Subduction Zones, areas of the earth's crust that are sinking beneath other portions of the crust, causing the surrounding tectonic plates to shift, forming mid-ocean ridges (Stern, 2002). Volcanic arcs are found all over the world, but the majority are in the western Pacific Ocean with attached geothermal systems (Trujillo & Thurman, 2008). They are also above land, for example, Central Andes Volcanic Zone (Grosse et al., 2009). Volcanic arcs allow terrestrial geothermal systems to be found in the middle of the ocean (Stern, 2002).

Calderas are 1–100 kilometre depressions that are most likely caused by an eruption, where the volcanic cone collapses due to pressure build-up in the magma chamber (Acocella, 2007). These eruptions often lead to tectonic unrest, significantly altering the surrounding areas and local climate change, but do not necessarily cause the extinction of the volcano (Lowenstern et al., 2006). Caldera formation can vary, for example, the most well-known caldera is Yellowstone National Park in the United States, which formed suddenly approximately two million years ago whereas, Bárdarbunga, in Iceland, collapsed over the period of several months in 2014 (Gudmundsson et al., 2016;

Lowenstern et al., 2006). Calderas are unique volcanic environments that lend themselves to a multitude of scientific studies.

Geothermal hotspots are areas of fluctuating heat near a source of magma that is not located near plate boundaries that are caused by mantle plumes that have risen above sea level and generally have considerable volcanic activity (Henley & Ellis, 1983; Trujillo & Thurman, 2008). Hotspots are surrounded by geothermal fields, which are areas where water that has been heated by the lower mantle has travelled through cracks to the surface (Breeze, 2019; Trujillo & Thurman, 2008). This usually comes in the form of hot springs, fumaroles, geysers, hot mud pools, and volcanoes (Breeze, 2019; Trujillo & Thurman, 2008). There are currently dozens of active hotspots around the world, the most well-known of which are islands such as the Hawaiian Islands, Deception Island, and Iceland, mid-continental locations such as Yellowstone National Park, and volcanoes such as Mt. Erebus and Mt. Melbourne (Hurwitz & Lowenstern, 2014; *Management Plan For Antarctic Specially Protected Area No. 175*, 2014). Hotspots allow geothermal areas to be found outside of tectonic plate boundaries.

Hotspot Volcanoes are formed above hot spots that are located in the asthenosphere (Devey et al., 2003). Mantle plumes bring material to the surface, and convection spreads the material laterally, while repeated action spreads the material vertically (Devey et al., 2003). Current hotspot volcanoes can be located on the hotspot or shifted away due to plate tectonics, as in the case with the Hawaiian Islands (Devey et al., 2003; Trujillo & Thurman, 2008). Another well-known hotspot is the volcano Mt. Erebus in Antarctica that is actively ejecting gas from the crater (Global Volcanism Program, 2020). Hot spot volcanoes can be formed in a variety of ways and are found around the world (Trujillo & Thurman, 2008).

1.2 Microbial communities in geothermal areas

1.2.1 Biogeography

Biogeography examines the theory that biota is found across the world in all types of environments such as polar environments, industrial regions, deserts, and even geothermal environments (Fierer et al., 2009). Baas-Becking acknowledged this when he

stated, “everything is everywhere, but the environment selects” (Baas+becking & Nicolai, 1934). Geothermal systems have elevated temperatures and are often associated with physico-chemical extremes due to their proximity to magma, such as the soil communities found on Mt. Erebus, Antarctica (Henley & Ellis, 1983; Herbold, Lee, et al., 2014; Soo et al., 2009). The geochemical and specific characteristics of these systems create specific niches where adapted organisms can thrive.

1.2.2 Taupō Volcanic Zone

The Taupō Volcanic Zone in the central North Island of New Zealand is 250 kilometres long, located near the subduction zone of the Pacific and Indian plates. This includes the volcanic island, White Island (Bibby et al., 1995) (**Figure 1.2-1**). The volcanic activity in the zone contributes to high heat flow creating many geothermal fields that contain geothermal springs. Recent studies suggest that the temperature and pH of these springs are important in creating unique ecosystems in which the microbial communities present can live (Power et al., 2018). Studies have shown the area has reduced microbial richness compared to some non-geothermal regions (Power et al., 2018). The bacteria shown to survive in these regions include the phyla: Aquificae, Proteobacteria, and Deinococcus-Thermus (Power et al., 2018).

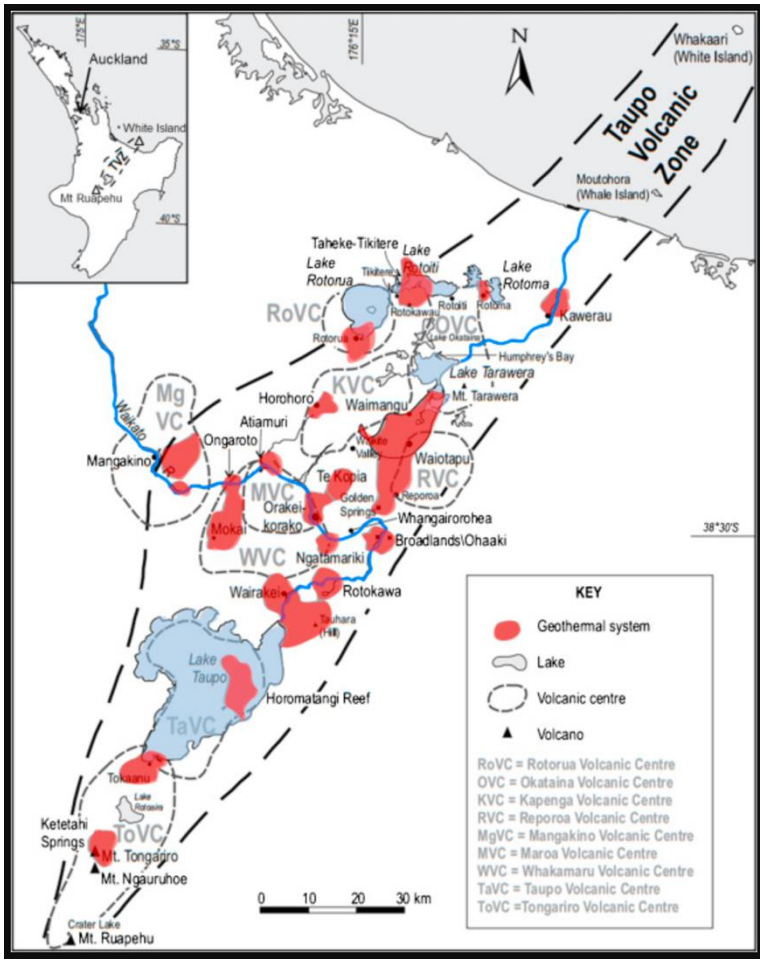

Figure 1.2-1: A Map of the Taupō Volcanic Zone sourced from Ratouis & Zarrouk (2016).

1.2.3 Yellowstone National Park

Yellowstone National Park is a volcanic caldera characterised by geothermal springs that are frequently changed by volcanic eruptions (**Figure 1.2-2**) (Takacs-Vesbach et al., 2008). The volcanic caldera was formed by several eruptions that occurred 2.0, 1.3, and 0.6 million years ago (Fournier, 1989). The caldera is still active today, as evidenced by the 350 °C temperature in the shallow regions, the decreased rock density, continued seismicity, and the increased convective and conductive heat flow (Fournier, 1989; Hurwitz & Lowenstern, 2014; Shelly et al., 2013).

The microbial communities in the geothermal areas of Yellowstone National park have been changed by volcanic eruptions, which may have caused ancestral microbes to go extinct as well as interrupted dispersal of the bacteria across Yellowstone National Park

(Takacs-Vesbach et al., 2008). The distribution of the microbial communities depends on the current environment as well as past history of volcanic eruptions and dispersal events (Takacs-Vesbach et al., 2008). Within the caldera, there are endothilic microbial communities that thrive in low pH conditions at approximately 35 °C (Takacs-Vesbach et al., 2008). Thermal communities consisted of photosynthetic algae as well as *Cyanidium*, Aquificales, *Mycobacterium*, Chloroflexi, Nitrospirae, and Planctomycetes (Takacs-Vesbach et al., 2008; Thiel et al., 2016). Yellowstone National Park is home to many unique microbial communities that drive research in this area.

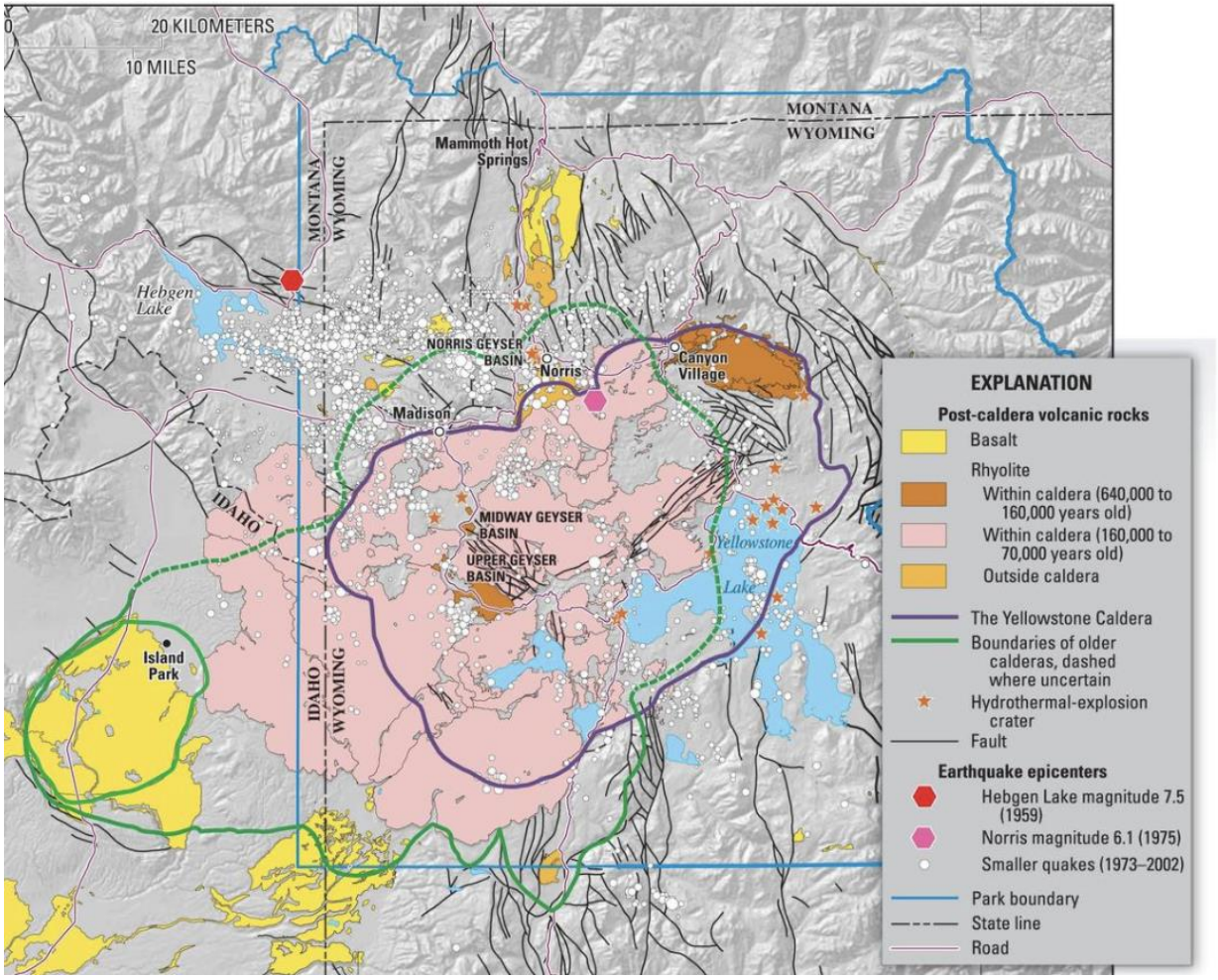

Figure 1.2-2: A Map of The Yellowstone Calderas sourced from USGS (https://www.usgs.gov/media/images/simplified-map-yellowstone-caldera)

1.2.4 Mt. Erebus

Mt. Erebus on Ross Island in Antarctica has terrestrial geothermal systems set up around carbon dioxide vents (Herbold, McDonald, et al., 2014). The surface of Mt. Erebus has three craters and numerous hot spots that contain active (steam emitting) and passive (not steam emitting) fumaroles—an opening on the surface of a volcano that emits carbon dioxide and steam, creating a constantly warm geothermal environment (Herbold, Lee, et al., 2014). Studies of these hot spots have looked at soil temperature and pH and found unique environments that are able to house unique biota due to specific physico-chemical properties (Herbold, Lee, et al., 2014; Soo et al., 2009).

Genetic studies looking at this biota have found endemic bacteria species such as Chloroflexi, Acidobacteria, and Planctomycetes, as well as cosmopolitan bacteria species such as Actinobacteria, Cyanobacteria, Firmicutes, Deinococcus and Meiothermus (Herbold, Lee, et al., 2014; Soo et al., 2009). It is thought that the cosmopolitan bacteria are most likely related to the biota of South America due to land proximity and wind patterns (Herbold, Lee, et al., 2014). There have also been similarities observed between the biota found on the northern Antarctic islands and Mt. Erebus, suggesting aeolian distribution (Herbold, Lee, et al., 2014; Soo et al., 2009; Torres et al., 2011). Overall, previous studies have found that the microbial communities on Mt. Erebus are composed of endemic and cosmopolitan bacteria (Herbold, Lee, et al., 2014).

1.3 Thermophiles

1.3.1 Defining Characteristics of Thermophiles

Thermophiles are organisms found in environments such as geothermal areas where the temperature is typically between 40 to 79 °C, with a subclass called hyperthermophiles that thrive at 80 – 120 °C (Gottschal & Prins, 1991) (**Figure 1.3-1**). While thermophiles are found in every domain of life, Archaea have the highest tolerance for temperature, followed by bacteria, then eukaryotes (Straub et al., 2018; *Thermophilic Communities - Yellowstone National Park (U.S. National Park Service)*, n.d.; *Thermophilic Eukarya - Yellowstone National Park (U.S. National Park Service)*, n.d.). Thermophiles in these communities produce valuable products for other members of the community, including fixed carbon and nitrogen (Straub et al., 2018)

A diverse range of bacteria have adapted to harsh conditions in order to thrive in unique environments (Sammond et al., 2016). The first thermophiles to be isolated and studied were spore-forming bacteria, in particular, *Bacillus strearothermophilus* (T. D. Brock & Freeze, 1969). Thermophilic bacteria are the most diverse of the three branches of the tree of life and have driven thermophilic research forward (Thomas D. Brock, 1997). As research continues, more thermophiles with various mechanisms of survival and function continue to be found. (Thomas D Brock, 1967).

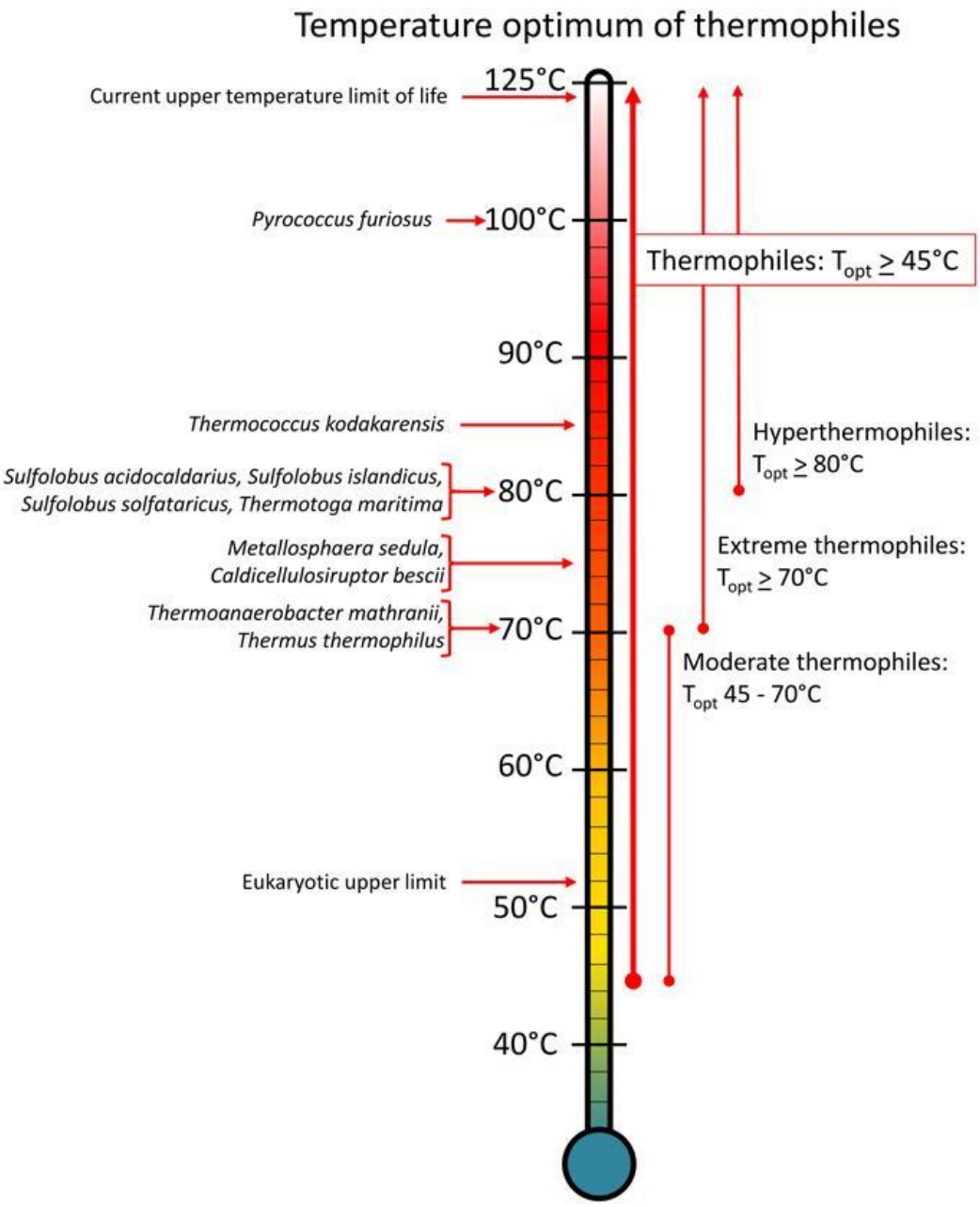

Figure 1.3-1: The Temperatures at which well-known thermophiles thrive sourced from Zeldes et al., 2015

1.3.2 Environments for Thermophiles

Thermophiles exist all over the world in geothermal environments. Specifically, studies of Yellowstone National Park have had great significance for thermophilic research (Takacs-Vesbach et al., 2008). The more than 10,000 geothermal spots are home to numerous thermophiles, including the most famous, *Thermus aquaticus,* which was isolated from Mushroom Spring in 1969, a 60 °C hot spring with microbial mats, and has advanced molecular biology (T. D. Brock & Freeze, 1969; *Hydrothermal Features - Yellowstone National Park (U.S. National Park Service)*, n.d.; Thiel et al., 2016).

There are large geothermal regions around the world that have influenced the knowledge of thermophiles and microbial communities. Taupo Volcanic Zone in New Zealand is another geothermal region that is home to unique thermophiles that live within approximately 1,000 hot springs that stretch across a geothermal field almost the entirety of the central North Island (Power et al., 2018). Geothermal regions can provide warm islands within cold environments such as Antarctica (Fraser et al., 2014). Thermophiles have been found in the hot springs of Deception Island as well as other nearby islands and in the terrestrial geothermal fields of Mt. Erebus, Ross Island (Herbold, Lee, et al., 2014; Soo et al., 2009; Torres et al., 2011).

1.3.3 Adapting to extreme environments

Many microorganisms display a temperature range preference for functionality, as well as adaptation to use specific substrates for survival. Many thermophiles produce products that can be used to support other members of the microbial community and for biotechnology purposes (Thomas D Brock, 1967; Gottschal & Prins, 1991; Juergen Wiegel et al., 1985). These microbes can produce sulphuric acid, hydrogen sulphide, methane, and carotene (T. D. Brock & Freeze, 1969; Jürgen Wiegel et al., 1981; Jürgen Wiegel & Ljungdahl, 1981). The ability to process substrates found in geothermal regions aids them in surviving the harsh conditions.

The membranes of thermophilic bacteria have a unique structure compared to mesophilic bacteria, organisms that prefer temperatures of 20–45 °C (Goldstein, 2007; Shilo & Hirsch, 1979). Thermophilic cell membranes have a higher population of saturated and straight-chain fatty acids in order to maintain membrane fluidity at extreme temperatures (Shilo & Hirsch, 1979). These mechanisms for maintaining the stability of DNA does not depend on the GC content but rather the ability to prevent mutations (Shilo & Hirsch, 1979; Q. Wang et al., 2015). The proteins present within thermophiles do not unravel at high temperatures, unlike some other life forms. Kelch & Agard (2007) examined the structure of mesophilic and thermophilic proteases to analyse what structural features enables the protein to maintain stability. They found that there was a domain bridge that increases the surface area side chain interactions and significantly slows the unfolding of the protein by stabilising the kinetics and changing the secondary structure (Kelch & Agard, 2007). Thermophiles most often adapt to high temperatures by increasing hydrogen bonds, hydrophobic interactions, and salt bridges (Razvi & Scholtz, 2006). Mutations are strategic in thermophiles; single missense mutations can be advantageous to the thermophiles, but overall the proteins more tightly folded than in mesophiles to avoid mutations (Razvi & Scholtz, 2006). These adaptations aid thermophiles in surviving at high temperatures.

1.3.4 Importance of Thermophiles

Thermophiles are important to the continuation of biogeochemical cycles, major elements processed by microorganisms transforming them to be used by other organisms in nature (Zhao et al., 2014). Terrestrial thermophiles have been sequenced, showing key enzymes involved in nitrogen; for example, Phyla such proteobacteria and Chloroflexi play key roles in the oxidation of nitrates (Spieck et al., 2020; Zhao et al., 2014). Additionally, Acidobacteria metabolising both organic and inorganic nitrogen (Eichorst et al., 2018). Other thermophiles such as Deinococcus participate in carbon fixation (Ferreira et al., 1997). Thermophiles, for example, those in the Acidobacteria, are also important in the reduction of elemental sulphur to hydrogen sulphide in the biogeochemical cycling of sulphur (Kalam et al., 2020). Thermophiles fill an important role in the processing of elements in the environment(Zhao et al., 2014).

1.4 Geothermal Microbial Community Drivers

Microbial communities across the world and in every environment are shaped by physiochemical features such as temperature, pH, salinity, and nutrient availability (Soo et al., 2009; S. Wang et al., 2013). These factors will influence what biota will be both present and active within the community; however, these drivers are not well understood (Chiriac et al., 2017; Soo et al., 2009; Takacs-Vesbach et al., 2008; S. Wang et al., 2013). Further in-depth studies are needed to have a greater understanding of the relationship between the microbial communities, the environment, and how these physico-chemical features drive community structure.

1.4.1 Temperature

Temperature gradients are found worldwide in geothermal systems, e.g. Iceland, the Tibetan Plateau, China, Sungal Klah, Malaysia, Chiraleu, Ciocaia, and Mihai Bravu, Romania, Yellowstone National Park in the United States, and Taupo, New Zealand (Chiriac et al., 2017). Most well-studied thermal gradients are found in hot springs, such as Yellowstone National Park. Hot soils are less well studied; examples of these are volcanoes found on Ross Island in Antarctica (Chiriac et al., 2017). The microbes present in these communities fulfil unique jobs and have adapted to live within the temperature gradient, but not all of the biota present in the community contribute to it (Rittershaus et al., 2013). When conditions are at a less than optimal temperature, the microorganisms remain in the community but arrest their growth or form spores (Rittershaus et al., 2013).

Microbial communities found within thermal gradients follow general trends. For instance, it has been observed that alpha diversity and richness of the samples decreases with an increase in temperature (Chiriac et al., 2017; Tobler & Benning, 2011). Studies have shown how the relative abundance of Chloroflexi increased as the temperature increased, leading to a shift in community structure, suggesting that temperature plays a key role in the clustering and structure of the communities (Chiriac et al., 2017; Tobler & Benning, 2011; S. Wang et al., 2013).

1.4.2 pH

In many studies, pH has been shown to be a critical factor in defining the structure of a microbial community (Soo et al., 2009). Many bacteria have a tolerance for a specific pH range that dictates where they are able to thrive (Jiang & Takacs-Vesbach, 2017). Globally, pH shapes soil communities across a wide variety of environments, including hot springs, urban soils, arctic environments, and alpine environments (Jiang & Takacs-Vesbach, 2017; Purcell et al., 2007; Soo et al., 2009; Zhalnina et al., 2015). The pH of these environments is affected by outside factors such as weather, fertiliser application, mineral deposits, and human interventions (Zhalnina et al., 2015).

pH has been shown to be one of the strongest influencers of microbial communities, particularly at the phylum and genus levels, with the highest microbial diversity found at neutral pH (Zhalnina et al., 2015). They hypothesised that the pH is the strongest influence on a system due to pH facilitated nutrient availability, thus allowing the system to thrive or allow pH dependent enzymes to function properly. A lower pH leads to a higher ratio of carbon and nitrogen as well as a difference in the ion concentration, thus leading to environmental differences between sites (Jiang & Takacs-Vesbach, 2017; Zhalnina et al., 2015). pH and temperature have the most influence within pH gradients (Jiang & Takacs-Vesbach, 2017; Purcell et al., 2007; Zhalnina et al., 2015).

1.4.3 Salinity

While pH and temperature are often the main sources of influence on microbial community structure, salinity also plays a key role in the composition of a community (Lozupone & Knight, 2007; Oren, 2015). Salt concentration has differing effects on the community depending on the substrate and environment in which it is found (Rath & Rousk, 2015). When the salt concentration of an environment is greater than 35 %, it is considered extreme (Boetius & Joye, 2009). Salinity is a defining component of deep-sea brines, salt lakes, evaporation ponds, soils, and hot springs (Boetius & Joye, 2009; Bougouffa et al., 2013; Oren, 2015). Well-known examples are the Red Sea, Mediterranean Sea, and the Dead Sea, but in Antarctica, there are several lakes with high salt concentration: Organic Lake and Deep Lake (Boetius & Joye, 2009; Oren, 2015; Yau et al., 2013). There is also Blood Falls in the Dry Valleys, Antarctica that is an iron filled brine that drains from Taylor Glacier (Boetius & Joye, 2009). Studies researching

microbial communities in these areas have shown that the high salt concentration shapes the diversity of the biota present in the environment (Oren, 2015).

There are a number of halophiles, organisms that live in high salt concentrations, across the tree of life (Boetius & Joye, 2009; DasSarma & DasSarma, 2017). In order to function in high salt environments, microbes make a number of adjustments to their cellular structure; in particular, their enzymes are adapted, and intercellular solutes are concentrated (Oren, 2015). These hypersaline environments have varying pH's but often have high sulphate and sulphide concentrations, promoting the survival of biota that are able to reduce sulphate (Oren, 2015). Sass et al. (2001) showed a correlation between the presence of chlorine and other elements and an increase in the diversity of a geothermal community despite other unfavourable conditions. Additionally, increased salt concentration has been shown to be associated with increased diversity of microbial communities present in brine pools (Bougouffa et al., 2013; Heijs et al., 2008).

1.4.4 Carbon and Nitrogen

Carbon and nitrogen are essential for microbial growth and are considered limiting growth factors (Aldén et al., 2001). Stoichiometric proportions of these elements are critical to the composition of microbial communities (Drenovsky et al., 2004). Organic carbon often selects for the microbes that are able to absorb it the fastest. The carbon to nitrogen ratio present in the soil is influenced by the ratio of fungus to bacteria. The higher the ratio of fungus:bacteria the higher the carbon:nitrogen ratio due to the fungi's slow processing of the nitrogen (Bowles et al., 2014; de Vries & Bardgett, 2012; Fierer et al., 2009).

The elements crucial to the survival of life have an effect on the microbial communities. On Deception Island, for example, it has been observed that the communities present at fumaroles have an increase in carbon in the environment that has led to a decrease in the number of OTUs, and the community structures appear more influenced by temperature (Bendia et al., 2018). While Jorgensen et al. (2012) found microbial communities present in the mid-ocean ridge have been shaped by organic carbon, iron, manganese, and sulphate concentration.

Collectively these drivers (temperature, pH, salinity, carbon, and nitrogen) shape the communities in unique ways. Most studies on these drivers, particularly the ones that were done in geothermal environments, have focused on hot springs, whereas the geothermal gradients of this study are polar high-altitude terrestrial environments. While these studies provide important information on how drivers influence and shape communities, it is still unknown how they influence microbial communities.

1.5 Antarctica

1.5.1 Shaping of a Continent

Antarctica began to break away from the supercontinent, Gondwana, in the early cretaceous period, approximately 136 million years ago (Casey, 1964). Magmatism and microplate movement allowed Antarctica to move away from its spot between Australia and Africa. There are five tectonic regions that make up the 14.2 million km^2 of Antarctica. (*Antarctica - The World Factbook*, n.d.; Storey et al., 1988). These align with the geothermal features and explain the similarities across the continent that took shape over many millions of years (Goodge, 2020). The Cenozoic Era saw the critical shaping of Antarctica, as this was when it moved to the bottom of the planet, shifting the climate to a new polar environment (Storey et al., 1988). The late Cenozoic Era, beginning 66 million years ago, created the Transantarctic Mountain Range that stretches from peninsular Antarctica to Cape Adare and includes Mt. Erebus, Mt. Melbourne, and Mt. Rittmann (Davies et al., 2012; Lemasurier & Wade, 1968) (**Figure 1.5-1**). In addition to the mountain ranges, the Western Antarctic Rift System is responsible for the shaping of the continent (Goodge et al., 2020). The rift system caused the spreading of the continent and bringing the continent closer to the earth's magma layer and thus increasing the geothermal activity in the region (DiVenere et al., 1994).

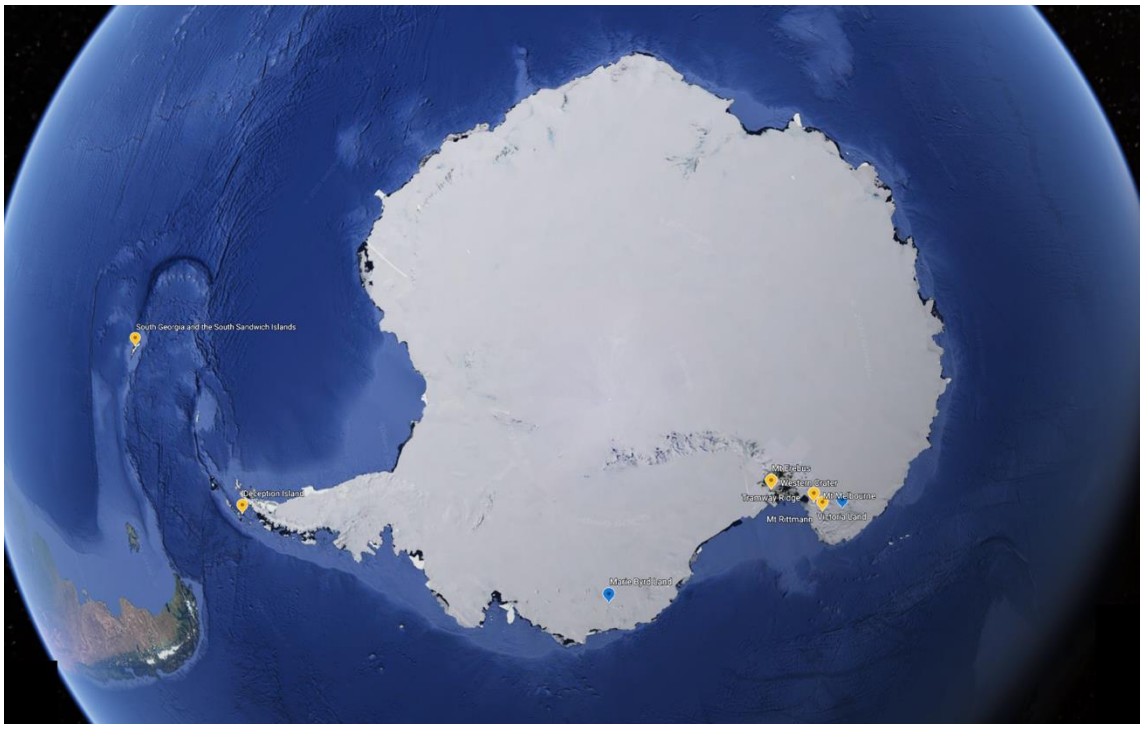

Figure 1.5-1: A Labelled Map of Antarctica sourced from Google Earth

Antarctica is one of the most unique environments on our planet, thus it is one of the most valuable environments for scientists (Boyd et al., 1966). Due to the harsh conditions present and the remote nature of the continent, most of Antarctica remains undisturbed by humans. Tectonic plate movement has led to the development of microclimates such as the desert of the Dry Valleys and the moisture rich Deception Island (Bendia et al., 2018; Cary et al., 2010; Smith, 2005). The geothermal regions created by the movement of the rift system allows thermophiles to thrive in Antarctica (Fraser et al., 2014). Antarctica consists of continental Antarctica and the surrounding islands, which fall within the northern latitudes. The geographical nature of the islands allows for much warmer environments than is possible on continental Antarctica (Convey & Lewis Smith, 2006).

Antarctica is characterised by a number of unique features that seem unlikely to harbour life, but nevertheless, they are home to a diverse group of flora and fauna, including birds, seals, mosses, cyanobacteria, bacteria, fungi, and more (Convey et al., 2000; Soo et al., 2009; R. H. Taylor et al., 1990.). Volcanic Islands are scattered throughout the ocean

surrounding the continent. The Transantarctic Mountains divide the East half of Antarctica from the West half (Goodge et al., 2020). There are geothermal hotspots as well as volcanoes scattered throughout the continent (Herbold, McDonald, et al., 2014). The border of the continent also contains many ice shelves (Fretwell et al., 2013). The continent has the Dry Valleys on the coast of Victoria Land, at one time thought to be unable to support life, but which research now shows is not true and that even these harsh conditions can support diverse flora and fauna (Cary et al., 2010).

1.5.2 Geothermal Systems in Antarctica

Geothermal systems across Antarctica provide a unique environment for a diverse range of biota across this harsh continent, even allowing species to survive that are not often associated with polar regions (Herbold, McDonald, et al., 2014; Soo et al., 2009). This is due to the fact that plate tectonics have created areas where magma is brought close to the surface, thus bringing warmth and freely available water to an otherwise freezing and dry environment (Trujillo & Thurman, 2008).

Geothermal regions and nunataks (ice free regions) are areas of refuge that allow high species diversity and richness to survive through hardships such as glacial maxima and everyday life in a polar environment (Fraser et al., 2014). The mainland, the peninsular region, and coastal regions are all reported to differ in the composition of micro and macro flora present (Convey & Lewis Smith, 2006). The plant life here varies with the temperature of the geothermal regions (Soo et al., 2009). In recent history, the pattern and placement of geothermal systems has stayed constant, following the patterns created by the tectonic plates (Convey & Lewis Smith, 2006). Events like volcanic eruptions have interrupted the pattern and killed off the associated flora and fauna (Takacs-Vesbach et al., 2008). This is observed across the continent but is particularly noticeable in close proximity to the Transantarctic Mountains or on nearby islands surrounding the continent (Fraser et al., 2014). Not only is there increased richness of microbial life, but there is also increased presence and diversity of vegetation due to the temperature gradients present at these geothermal sites (Herbold, McDonald, et al., 2014). The geochemical drivers can not only be influenced by the temperature of the system but also by the size of the geothermal fields (Fraser et al., 2014). The movement of biota via water, wind, and

human movement can transfer species between geothermal systems. This means the biota of one system is not necessarily isolated from that of another. The lightweight nature of moss spores allows them to be spread across systems with great ease. Fraser et al. (2014) has shown phylogenetically similar species of mosses found hundreds of kilometres apart. Studies suggest lichens also show increased diversity within geothermal regions, but it is less common than in mosses (Convey & Lewis Smith, 2006).

The role of tectonic activity in creating geothermal systems is evident in the variety of Antarctic plant life that varies with the temperature of the geothermal regions. Geothermal regions and nunataks are high in species richness. The mainland differs from the peninsular region, and both regions vary in relation to coastal regions. For example, nunataks allow for a high diversity of invertebrates to survive, but there are differences in the communities of coastal and inland nunataks (Fraser et al., 2014). This is likely because their geothermal activity has created an environment of warmth where species can survive through various cycles of climate change across millennia. Each geothermal region in Antarctica has its own unique community structure influenced by the tectonic changes. Fraser et al. (2014) argue for the study of these regions to understand the full breadth of the diversity in the geothermal landscape. Prominent geothermal systems include Deception Island, South Sandwich Islands, Marie Byrd Land, and Victoria Land.

1.5.3 Deception Island

Deception Island is a small horseshoe shaped volcanic island 17 km wide and 542 m above sea level (m.a.s.l). It is part of the South Shetland Islands and is just off the tip of the Antarctic Peninsula (Llarch et al., 1997). The island is mostly ice free and volcanically active, with temperatures reaching above freezing, with an average temperature range of -10 °C to 2.5 °C (Smith, 2005). Volcanic eruptions have led to the creation of new geothermal regions on Deception Island. These were colonised rapidly by phylogenetically related strains and exotic species of mosses (Convey & Lewis Smith, 2006). These exotic species are thought to have been spread by aeolian distribution from other Antarctic environments, as well as other continents. Nine sites were surveyed in 2002 and illustrated the uniqueness of Deception Island compared to the rest of Antarctica, with the exception of the nearby South Sandwich Islands (Smith, 2005). Surveys of the flora present showed that the majority of mosses and liverworts were dependent on the

warmth from the geothermal regions for survival, while 20 % of the mosses showed no reliance on warmth (Smith, 2005). The bryophytes on Deception Island are critical to the ecosystem (Smith, 2005). Bryophytes allow for carbon and nitrogen cycling in these communities via photosynthesis and form symbiotic relationships with cyanobacteria, allowing the fixed carbon and nitrogen to be used by the bacteria in the community (Turetsky, 2003). The springtail species *Collembola* is found only on Deception Island (Greenslade et al., 2012). It is thought the geothermally heated soils and moisture present allow the species to thrive much farther south than they would normally be found. Greenslade et al. (2012) suggest that the diversity of biota on Deception Island has been affected by human presence, leading to the introduction of non-indigenous species and hindering the proliferation of indigenous species. In addition to the bryophytes present, there are also thermophilic bacteria found in the geothermal soils, including the genera *Geobacillus, Bacillus, Brevibacillus,* and *Thermus* (Torres et al., 2011). Llarch et al. (1997) found *Bacillus* species, a mesophilic bacteria, that had adapted to live in the thermal environment within Deception Island. Strains of bacteria have adapted to live within the many temperatures on Deception Island. They found strains that can live in an extended temperature range that varies per species. On average, the temperature ranges from 16–62 °C.

1.5.4 The South Sandwich Islands

The South Sandwich Islands consist of 11, 1–3 million-year-old islands that are located on the northern side of the Antarctica latitude boundary, 60°S (Convey & Lewis Smith, 2006). Their position south of the Polar Frontal Zone means the climate is characteristically harsh (Convey et al., 2000; Convey & Lewis Smith, 2006). There are several active volcanoes scattered across the islands, with many fumarole sites near the main crater of these volcanoes (Convey et al., 2000). These fumaroles are characterised by steep temperature gradients spanning 20–75 °C. Many of these fumaroles and surrounding areas have been examined for bryophyte life and contain stratified bryophytes and microbial communities (Convey & Lewis Smith, 2006). A study of maritime biota has shown that 50 % of the mosses and 89 % of liverworts are dependent on the heat from the geothermal regions to survive (Convey & Lewis Smith, 2006). The quality of life of these bryophytes exponentially decreased outside of the geothermal environments (Convey & Lewis Smith, 2006). There are appears to be a limited

geographic range for these marine bryophytes, as those that live on the periphery of the geothermal soils are still on warm soil that is not actively heated (Convey & Lewis Smith, 2006). Four of the surveyed mosses did not require a geothermal environment (Convey & Lewis Smith, 2006). Of the terrestrial mosses, four were only found in Antarctica, 15 were found in places around the Southern Hemisphere, and 17 were found all over the world, including the Arctic islands (Convey et al., 2000). The basidiomycetes and lichens observed are local organisms, the majority found in Antarctica, with no species being endemic to the South Sandwich Islands (Convey et al., 2000). Most of the terrestrial liverworts studied showed phylogenetic similarity with species from South America (Convey et al., 2000). Surveys were conducted across the islands near the Antarctic peninsula to determine the similarity of the biota in particular bryophytes, 13 of the bryophytes were found across the islands. (Convey et al., 2000). The terrestrial flora found on the South Sandwich Islands is markedly similar to the flora found on Deception Island (Convey et al., 2000). *Campylopus pyriformis* was found on these islands, the only other known place where this moss is found is on Mt. Melbourne and Mt. Erebus (Skotnicki et al., 2001). The reproductive pattern of these mosses allows them to be cosmopolitan no matter whether the environment is terrestrial or marine (Skotnicki et al., 2001). The South Sandwich Islands are particularly dominated by *Bacillus* species, including *B. shackletonii* and *B. luferensis,* which are both known to be found in volcanic soil ((Logan et al., 2002, 2004)

1.5.5 Marie Byrd Land

Marie Byrd Land is a remote region approximately 720 km long, and 320 km wide in western Antarctica characterised by two mountain ranges with volcanoes dispersed amongst the mountains (Lemasurier & Wade, 1968; Wysoczanski et al., 1995). The environment of Marie Byrd Land is characterised by high wind speeds and recently formed high volcanoes (Lemasurier & Wade, 1968) that form part of the Cenozoic volcanism belt (Wysoczanski et al., 1995). The volcanoes have active fumaroles and open vents that are alkaline in nature (Lemasurier & Wade, 1968). Most studies conducted on Marie Byrd Land have focused on the formation of the volcanoes rather than the organisms that are able to thrive in the volcanically warmed soils (*Management Plan For Antarctic Specially Protected Area No. 175*, 2014).

1.5.6 Victoria Land

Victoria Land, located in West Antarctica on the coast of the Ross Sea, stretches from 72°S to 76°S and has three active volcanoes: Mt. Rittmann, Mt. Melbourne, and Mt. Erebus. Mt. Rittman is the most recently discovered volcano of the group, found in the 1980s. The accessibility of Mt. Melbourne has allowed it to become a highly studied system (P.R. Kyle et al., 1990). Mt. Melbourne's surface contains vents, ice towers, and ice roofs (Lyon & Giggenbach., 1974). These volcanoes are well known for the temperature gradients observed within the geothermal systems. Mt. Erebus has been continually erupting since December 1972, making it the southernmost active volcano (Global Volcanism Program, 2020). The surface of Mt. Erebus is characterised by fumaroles and ice towers (Lyon & Giggenbach., 1974). Unlike the previous well-characterised areas, the geothermal regions of Victoria Land have limited water present. Organisms in Victoria Land are primarily dispersed using air currents (Lyon & Giggenbach., 1974; Herbold et al., 2014). Even with the limited methods by which to colonise Victoria Land, there are still bryophytes, algae, cyanobacteria, and bacteria present in the community (Broady et al., 1987).

1.5.7 Mt. Rittmann

Mt. Rittmann is one of the major active volcanoes in the Mountain Range in northern Victoria Land. It is 2,600 meters above sea level, a shield volcano, and approximately 100 kilometres from Mt. Melbourne (*Management Plan For Antarctic Specially Protected Area No. 175*, 2014). Preliminary research has been done across the volcanoes of Victoria Land. The fumaroles that cover the surface of Mt. Rittmann have ice free geothermal alkaline soils, high in concentrations of cadmium and lead (Bargagli et al., 1996). The surface temperature of the ice free soil reaches approximately 45 °C and is dotted with ice chimneys (*Management Plan For Antarctic Specially Protected Area No. 175*, 2014). These ice free soils are known to harbour mosses, algae, protozoa, and thermophilic bacteria (Bargagli et al., 1996).

1.5.8 Mt. Melbourne

Mt. Melbourne, a stratovolcano that is 2,733 m.a.s.l. It is estimated to have last erupted 150 years ago (*Management Plan For Antarctic Specially Protected Area No. 175*, 2014).

The surface of this volcano is covered with fumaroles, ice towers, and ice-free areas similar to Mt. Rittmann. These ice free areas are populated with bryophytes, algae, fungi, and bacteria, including *Bacillus, Klebsiella*, and *Micrococcus* (Broady et al., 1987). The diversity of bryophytes here is lower compared to the South Sandwich Islands and Deception Island (Convey et al., 2000). It is hypothesised that the soil on Mt. Melbourne and other Victoria Land volcanoes is much drier than other volcanoes in Antarctica, particularly the maritime volcanoes, leading to a smaller set of species that can live there (Convey et al., 2000). The geothermal regions allow water to be present in the soil and promote the adaptation of microorganisms to this specialised environment, and possibly lower diversity (Broady et al., 1987).

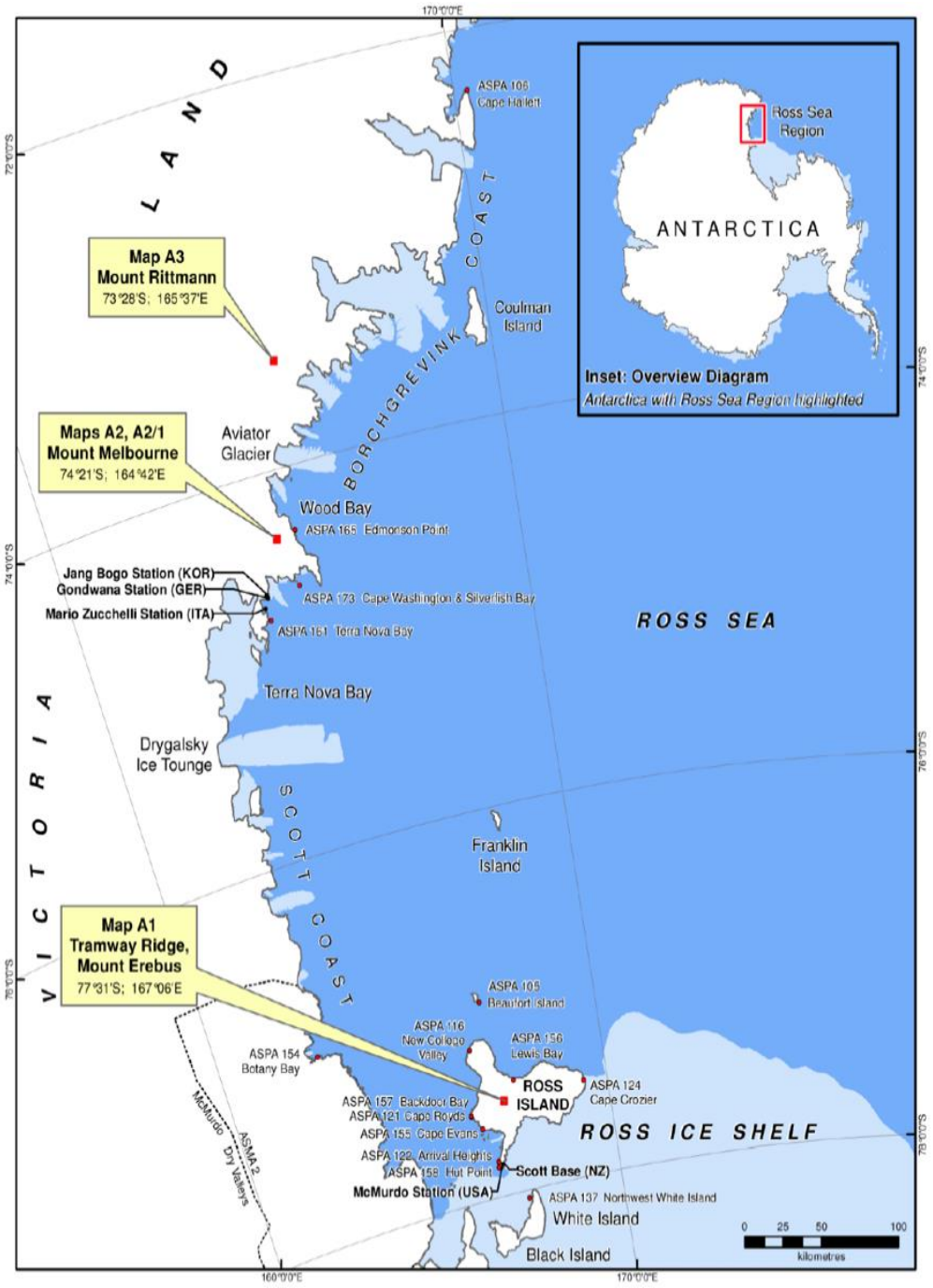

Figure 1.5-2: Map of Victoria Land including Mt. Rittman, Mt. Melbourne, Mt. Erebus including Tramway Ridge sourced from (*Management Plan For Antarctic Specially Protected Area No. 175*, 2014)

1.5.9 Ross Island

Ross Island is situated off the coast of Victoria Land in southern Antarctica and is a volcanically active island in the middle of the Ross Sea (Herbold, McDonald, et al., 2014) (**Figure 1.5-2**). It is home to four volcanoes, most notably Mt. Erebus (*Management Plan*

For Antarctic Specially Protected Area No. 175, 2014). In connection to the volcanoes, there are high altitude geothermal areas that differentiate Ross Island from the northern islands, such as the South Sandwich Islands and Deception Island (Sinclair & Sjursen, 2001). New Zealand's Scott Base and the United States' McMurdo Station are located on Ross Island leading to a higher human presence and impact on this island compared to other areas of the continent (*Management Plan For Antarctic Specially Protected Area No. 158 HUT POINT, ROSS ISLAND (Including Historic Site and Monument No. 18, the Historic Discovery Hut of Captain Robert Falcon Scott)*, n.d.). One of the main research focuses of Ross Island is the Adelie penguin colony - the ice free rocky regions of the island are perfect for these penguins (Taylor et al., 1990). In addition to the birds, most of the terrestrial flora and fauna communities are within close proximity of the shoreline where water is easily accessible, or in the ice free patches on the volcanoes where the steam from the vents condenses in the cold Antarctica air providing a water source for the organisms present (Sinclair & Sjursen, 2001). There are mites, tardigrades, springtails, algae, mosses, nematodes, rotifers, and protozoa (Sinclair & Sjursen, 2001). The communities on the volcanoes consist mostly of mosses, cyanobacteria, and thermophilic bacteria, including Proteobacteria, Acidobacteria, Chloroflexi, and Actinobacteria (Herbold, Lee, et al., 2014; Soo et al., 2009). There are relatively few studies done on the microbial communities on Ross Island outside of Mt. Erebus.

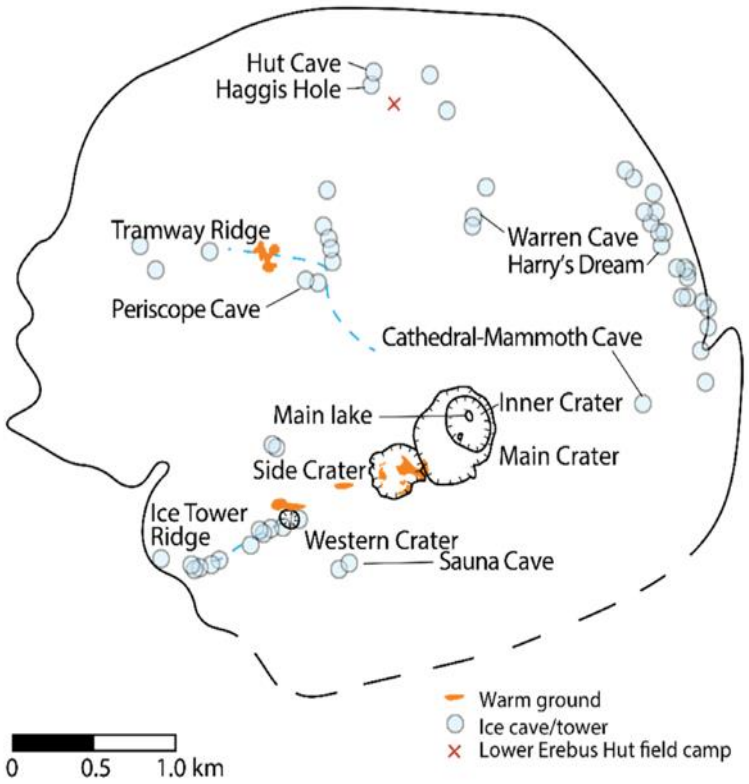

Fig. 2. Erebus caldera, with craters, major ice towers, and ice caves. Warm ground areas include areas around Tramway Ridge (dashed line), Western Crater, and Side Crater. Data from Panter and Winter (2008), Curtis and Kyle (2010), and field observations.

Figure 1.5-3: A Map of the Craters and Caves on Mt. Erebus sourced from (T. Ilanko et al., 2019)

1.6 Mt. Erebus

Mt. Erebus is the most prominent volcano on Ross Island, and is the tallest active volcano in Victoria Land at 3,794 m.a.s.l. It is a stratovolcano with three craters and a lava lake in its main crater (*Management Plan For Antarctic Specially Protected Area No. 175*, 2014). It has been continually erupting since December 16, 1972. (Global Volcanism Program, 2020). It features ice caves, ice towers, ice chimneys, caves, and ice free geothermal soils (Global Volcanism Program, 2020) (**Figure 1.5-3**). Mt. Erebus has been, and continues to be, an important site and focus for scientific discoveries in Victoria Land (Herbold, McDonald, et al., 2014). The ice free soils of Mt. Erebus are home to microbial life that lives at the base of the fumaroles on the surface (Herbold, Lee, et al., 2014; Soo et al., 2009; Tebo et al., 2015).

1.6.1 Caves

There are several caves on the surface of Mt. Erebus, including Warren Cave, Hubert's Nightmare, and Harry's Dream. These caves have a dark and warm environment with low nutrient levels and are thought to harbour low microbial diversity (Tebo et al., 2015). Warren Cave is the largest cave on the volcano has been used as a model system for dark caves (Connell & Staudigel, 2013; Tebo et al., 2015). Studies have shown that the microbial communities get their carbon source from volcanic emissions, which contain carbon dioxide, carbon monoxide, hydrogen gas, and various metals (Tebo et al., 2015). The vent location allows for the condensing of steam and water to flow into the cave bringing in nutrients (Tebo et al., 2015). Warren Cave is mostly populated by Actinobacteria and Chloroflexi (Tebo et al., 2015), while the phyla Basidiomycota and Ascomycota are the most prominent fungi present (Connell & Staudigel, 2013). By comparison, Hubert's Nightmare is a considerably smaller cave further from the main fumarole, and cooler in temperature, but higher in pH (Tebo et al., 2015). One phylum of interest present in the cave is Verrucomicrobia (Tebo et al., 2015). In 2012 the cave was frozen over, and it is thought the microbial community became inactive (Tebo et al., 2015). There is limited information on the microbial communities present. Harry's Dream receives light whereas the others do not (Tebo et al., 2015). The total organic carbon is significantly lower than the other nearby caves. Again, the nearest vent to the cave is not in an advantageous position, potentially leading to lower microbial diversity (Tebo et al., 2015). Cyanobacteria are the most prominent member of the microbial community in Harry's Dream. Unlike any other environment in Antarctica, there has been to date no archaea found in any samples analysed from Harry's Dream (Tebo et al., 2015).

1.6.2 Chimneys

Ice chimneys are a prominent feature on the surface of Mt. Erebus. They are formed on active volcanoes when steam released from a vent condenses and freezes due to a drastic difference in temperature between the atmosphere and the surface of the volcano. Cycles of this process lead to an accumulation of ice around the vent, and over many years' chimneys are formed (Herbold, McDonald, et al., 2014). There appears to be a connection between the formation of chimneys and the existence of caves (Herbold, McDonald, et al., 2014). The features on the surface of the volcano, including chimneys, are dictated by the flux of heat that the vents produce. The lower the heat flux, the easier it is for chimneys

to form (Herbold, McDonald, et al., 2014). Chimneys and steam release are a general indication of volcanic activity (Herbold, McDonald, et al., 2014).

1.6.3 Tramway Ridge

Across Mt. Erebus, there are geothermal fields that are home to much unique flora and fauna. The largest of these is Tramway Ridge which is approximately 1.5 kilometres away from the main crater (*Management Plan For Antarctic Specially Protected Area No. 175*, 2014). Within Tramway Ridge, there are a number of fumaroles at which the surface temperature can reach 65 °C (Soo et al., 2009). The field has temperature and pH gradients that decrease with distance from fumaroles. Mt. Erebus is recognised as a unique environment and is a specially protected area, ASPA (Antarctica Specially Protected Area) (*Management Plan For Antarctic Specially Protected Area No. 175*, 2014). This area is of great interest to scientists, but little research has been done on the microbial communities present in Tramway Ridge. The first study analysed the effect of pH gradients on the structure of the community, and showed that sulphur was the driver behind the shift in pH (Soo et al., 2009). 16S rRNA de novo sequence results showed which archaea and bacteria were present. The community structure differed significantly with proximity to the fumarole compared to away from the fumarole, with cyanobacterial mats in close proximity to the fumarole (Soo et al., 2009). This study showed that temperature, pH, and moisture are significant factors in structuring these microbial communities (Soo et al., 2009). Herbold, Lee, et al. (2014) showed that the diversity of the site decreased as the soil depth increased. Additionally, it was observed that diversity decreased as the activity of the fumarole decreased (Herbold, Lee, et al., 2014). Neither study was wide ranging, with a maximum of two fumaroles surveyed. These studies did not look at the physiochemical restraints on microbial communities present on the geothermal ridge (Herbold, Lee, et al., 2014; Soo et al., 2009). This study will look at three gradients at two different sites approximately one kilometre apart to achieve a broader picture of the geochemical drivers of the microbial community on Mt. Erebus.

1.6.4 Western Crater

Western Crater is another geothermal site located on Mt. Erebus, approximately a kilometre west of the main crater. It is poorly represented in scientific literature, with absolutely no microbial research done. The crater is affected by the wind more than

Tramway Ridge, and it is thought to be harder for larger flora such as mosses to survive there (Personal Communication Dr Craig Cary). This study will be the first to look in any depth at the microbial communities of Western Crater.

1.7 Molecular Tools for Microbial Analysis

1.7.1 CTAB

DNA extraction using Cetyl Trimethyl Ammonium Bromide (CTAB) will be the primary method used in this study. The CTAB protocol used was originally established by Murray & Thompson (1980). It has been widely used on many substrates, including previously difficult to extract samples such as Antarctic soil samples for many years (Barrett et al., 2006; Herbold, Lee, et al., 2014; Selkirk et al., 1998; Soo et al., 2009; Zhou et al., 1996). The version adapted for hot temperature low biomass soils by Herbold, Lee, et al. (2014) was used for this study.

CTAB extraction was developed as a safe and simple method that reduced the shearing of DNA and protein carry over, and allows DNA extraction in the presence of humic acid, which is often prevalent in environmental soil samples and known to cause inhibition in later steps of the DNA analysis process (Miao et al., 2014). Zhou et al. (1996) found that CTAB DNA extraction resulted in no loss of DNA. 23 kilobase long pieces of DNA are able to be extracted with limited damage, such as lower quality 260:280 ratio as well as with lower cost (Willner et al., 2012). CTAB extraction is more intensive and longer than other kit based DNA extraction methods, but generally, there is a high percentage of the DNA recovered (Sagar et al., 2014).

1.7.2 Ion Torrent

The Next Generation Sequencing technology that is used in this research is Ion Torrent sequencing. Primers from the Earth Microbiome project will be used to amplify a ~600bp fragment of the 16S genetic region with a barcode added for sample identification (Parada et al., 2016; Quince et al., 2011). Targeted sequencing of this well-known varied genetic region allows the identity of the microbes present in the communities to be known and analysed (Knight et al., 2018). This is a cost-effective method due to the small amount of

DNA that is required for amplification and sequencing, but it has a higher error rate that is known to create bias (Albertsen et al., 2015; Pollock et al., 2018). It is able to sequence up to approximately 400 base pairs and able to produce around 5 million reads depending on the sequencer. This is ideal for amplicon sequencing due to its small read length and quick sequencing time. The disadvantage of this method is it has an error rate of 1.5 erroneous bases per 100 bases compared to Illumina, which has an error rate of 0.8 erroneous bases per 100 bases (Loman et al., 2012). It requires 100-1000 ng, which is an average amount for sequencers per chip of 65 samples (Quail et al., 2012). However, there are sequencers that require more DNA, such as the PacBioRS (Quail et al., 2012). Each method has pros and cons that need to be considered. Ion Torrent is the cheapest and fastest method available (Quail et al., 2012).

1.7.3 R analysis

R is a computer language designed for statistical analysis based on previously developed languages, S and Scheme, that were combined in order to take advantage of the strengths of these languages to create a more useful language with a wider variety of tools to analyse the experimental data (Ihaka & Gentleman, 1996). The programming language is used within RStudio, a workbench designed to keep all the elements of R in one location (Allaire, 2012). Rmarkdown will be used within RStudio for a simplistic and readable (Baumer & Udwin, 2015; Ihaka & Gentleman, 1996). Rmarkdown aids researchers in sharing their results reputably by allowing for reproducibility (Baumer & Udwin, 2015). This presentation and syntax host many biological analysis pipelines to analyse the microbial diversity of the data by various methods (Allaire, 2012). This project will use R, including RStudio and Rmarkdown, to analyse 16S rRNA sequencing data results.

1.7.4 ASV

Amplicon sequence variants (ASVs) have been recently developed to better classify sequence data and to allow comparison of community composition across different studies (Benjamin J. Callahan et al., 2017). They were developed to replace operational taxonomic units (OTUs), which are sequences grouped based on 97 % genetic similarity (Westcott & Schloss, 2015). Conversely, ASVs are examined individually and are designed to use statistical analysis to discriminate on the basis of a single nucleotide to

determine the taxonomic classification of a DNA sequence. (Benjamin J. Callahan et al., 2019). Each taxonomic group is represented by an exact sequence in contrast to OTUs, which are represented by a consensus sequence of all the sequences present in the taxonomic group (Benjamin J. Callahan et al., 2017). An advantage of this method is that it is not reliant on a database to assign taxonomic labels to each sequence which frees it from reference; thus, it can be compared between studies, it has higher resolution, and the sequences represent exact biological sequences (Benjamin J. Callahan et al., 2017). The pipeline works by identifying the unique sequences that might be created by errors by using statistical analysis to determine confidence thresholds as well as considering copy number (Benjamin J. Callahan et al., 2017). An advantage of ASVs over OTUs is data can be added at a later point in time without changing the analysis of the data (Benjamin J. Callahan et al., 2017). ASVs do not solve the chimera problem, the fusion of two unique amplicons, within sequencing analysis, but ASVs do not introduce reference bias, depending on how they are analysed (Benjamin J. Callahan et al., 2019). Grouping sequences into ASVs allows statistical analysis between samples and studies due to the reference free grouping.

1.7.5 DADA2

DADA2 is an analytical tool used to generate ASVs, and it calculates the error rate and differentiates between PCR amplification errors and species sequences differences (Benjamin J. Callahan et al., 2014). The original Divisive Amplicon Denoising Algorithm (DADA) was developed to correct for these differences without assembling OTUs for 454 pyrosequencing (Rosen et al., 2012). The later version, DADA2, was developed in order to analyse amplicon Illumina sequencing (Benjamin J. Callahan et al., 2014). It has been successful at identifying taxa that differed by a single nucleotide. (Benjamin J. Callahan et al., 2014). It has been found to be more specific and more accurate than the most common methods currently used, such as UPARSE, QIIME, and MOTHUR (Benjamin J. Callahan et al., 2014). It also has the ability to detect fine-scale variation better than other methods (Benjamin J. Callahan et al., 2014). This pipeline identified more reference strains when compared to UPARSE though it is limited when identifying sequences that occur at lower frequencies (Benjamin J. Callahan et al., 2014).

1.7.6 Phyloseq

Ecological tools that could be applied to statistical data are available in R through Bioconductor, a collaborative and adaptive software system that can be edited for the researcher's needs (Gentleman et al., 2004). Phyloseq was developed to extend the capabilities of Bioconductor to analyse the phylogenetic relationships between the sequences found in the samples within R analysis (McMurdie & Holmes, 2013). While DADA2 assigns them into groups, Phyloseq establishes the relationship between the groups, taxonomy, and sample metadata (McMurdie & Holmes, 2013). Phyloseq takes ASVs data and outputs a richness plot using phylogenetic data (McMurdie & Holmes, 2013). Using phyloseq allows the evolutionary relationships between members of the microbial communities to be determined.

1.8 Applications, Future, Significance

This project examines the structure of the communities of microbes that live in the soil along geothermal gradients in Tramway Ridge and Western Crater, Mt. Erebus, Antarctica.

Next generation sequencing will be used to study the composition, structure, and diversity of the microbial communities (Archaea and Bacteria) that live within the multiple geothermal gradients of Tramway Ridge and, for the first time, Western Crater. Samples were taken along known geochemical gradients (Temperature and pH) to understand how geochemical parameters shape the microbial community structure and composition. Hence, this project will identify members of the microbial community within Western crater that were previously unknown as well as geochemical data of the soil within the crater. Collectively this will allow comparison of sequence data and geochemical analysis to establish the key driver/s of geothermal microbial communities in the island-like environment of Mt. Erebus.

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Chapter 2

An Investigation of Microbial Communities Across Two Extreme Geothermal Gradients on Mt. Erebus, Victoria Land, Antarctica

2.1 Introduction

Terrestrial geothermal systems are a common global feature that is often localised to tectonic plate boundaries or intra-plate hot spots (Trujillo & Thurman, 2008). These disparate features will often have extremes in physico-chemistry (pH, salt concentration, and metal composition) that characterise these geothermal sites (Henley & Ellis, 1983). Most of these geothermal systems support unique microbial communities highly adapted for these physiological extremes that continue to challenge our perception of the limits of life on the planet (Brock, 1967).

There is often a broad range of different geothermal features within any geothermal site, including hot pools, mud pools, hot soils, and steaming fumaroles. These features can vary in pH (2–10) (Jiang & Takacs-Vesbach, 2017), temperature (50–350 °C) (Wang et al., 2013), salinity (200–80,000 mg/kg) (Oren, 2015), with high concentrations of heavy metals, which are critical in shaping the composition and structure of active members of the resident microbial community (Henley & Ellis, 1983). The impact of an individual geothermal feature on its surroundings is very localised, often creating intense, highly compressed physico-chemical gradients (Power et al., 2018; Soo et al., 2009). Past research has shown that, most often, temperature, pH, and salinity are the most critical and primary drivers that shape these microbial communities (Chiriac et al., 2017; Jiang & Takacs-Vesbach, 2017; Lozupone & Knight, 2007; Power et al., 2018; Zhalnina et al., 2015). Collectively, these factors shape the community structure; however, the importance and interaction of each factor at each geothermal site is often poorly understood (Chiriac et al., 2017; Takacs-Vesbach et al., 2008; Wang et al., 2013; Zhalnina et al., 2015). Most geothermal research has been conducted in tractable temperate locations such as Yellowstone National Park, United States, Taupō Volcanic Zone, New Zealand, Bullicame Hot Springs, Italy (Power et al., 2018; Takacs-Vesbach et al., 2008; Valeriani et al., 2018). Only a few studies have explored geothermal microbial communities in polar systems.

Terrestrial polar systems are considered some of the harshest environments on the planet for life due to the perpetual cold, dry conditions with severely truncated seasonal solar input. There are only a few locations on the planet, mostly in the Arctic (Russia, Greenland and Iceland), where geothermal features can be found in a polar setting (Gudmundsson et al., 2016; Merkel et al., 2016; Roeselers et al., 2007). The Kamchatka Peninsula in Russia is currently one of the largest and hottest areas of volcanic activity located above the Arctic circle with features having temperatures over 90 °C and pH ranging from acidic to neutral, 3.5–7 (Bonch-Osmolovskaya, 2004; Gumerov et al., 2011; Merkel et al., 2016). Microbial surveys in Kamchatka have that the acidic pools are dominated by cyanobacteria and Archaea (Merkel et al., 2016). There are also a number of geothermal sites with microbial communities scattered across Greenland with temperatures up to 62 °C and pH from 7.0–9.5 (Roeselers et al., 2007). The hot springs in Iceland appear to resemble those in Yellowstone National Park, with temperature that ranges from 38–102 °C with an alkaline pH with pH 8 (Podar et al., 2020). In the southern hemisphere, Antarctica is home to many terrestrial geothermal sites, but it is largely unstudied in comparison to the Arctic (Herbold, McDonald, et al., 2014).

Thirty million years ago, the tectonic plates shifted, moving Antarctica from a connection with South America to its current more southern position; with this movement came a rich volcanic history and a shift from a temperate system to a true polar environment (Trujillo & Thurman, 2008). Only the remnants of the intense volcanism exist today (Boger, 2011; Casey, 1964). The remnants include the volcanoes on the Balleny Islands and the volcanic Sub-Antarctic Islands: Sandwich Islands and the collapsed volcano on Deception Island, (62° 57’S, 60° 38’W), which has an elevation 542 m above sea level (m.a.s.l) (Herbold, McDonald, et al., 2014). Deception’s fumarole sediments can reach temperatures as high as 100 ºC. Deception Island microbial communities appear to be driven by temperature and nitrogen concentration (Bendia et al., 2018). The Deception Island geothermal site is different from the rest of the continental sites because of a warmer climate, marine environment, and probable human contamination due to extensive visitation (Convey et al., 2000; Convey & Lewis Smith, 2006; Llarch et al., 1997; Torres et al., 2011).

There are three confirmed active continental geothermal sites, Mt. Melbourne (74° 21'S;164° 42'E), Mt. Rittmann (73° 28'S; 165° 37'E), and Mt. Erebus (77° 31'S; 167°06'E), all located in Victoria Land (Herbold, McDonald, et al., 2014). These continental volcanoes (Mt. Erebus, Mt. Melbourne and Mt. Rittman) have elevations higher than 2000 m.a.s.l and soil surface temperatures that can reach up to 65 ºC (Bargagli et al., 1996; Bargagli et al., 2004; Soo et al., 2009). It has been suggested

that geothermal areas in Antarctica have been vital for the survival of Antarctic terrestrial life during past glacial cycles, supporting the maintenance of diversity and enabling the later recolonisation of the continent (Fraser et al., 2014). This would support the hypothesis that these isolated geothermal systems could also support endemic microbial communities thriving in the sustained thermal gradients created between the hot and nearby cold soils (Herbold, Lee, et al., 2014; Papke et al., 2003; Takacs-Vesbach et al., 2008).

Mt. Erebus (3,794 m.a.s.l) is the southernmost active geothermal site in Antarctica. Mt. Erebus has been observed to be continuously active since December 1972 (Global Volcanism Program, 2020) and was also reported to have been active as far back as 1841 by Sir James Ross (R. Esser et al., 2004). The phonolithic lava lake in the main crater is the most notable feature on Mt. Erebus as it is only one of five active lava lakes in the world (Kyle, 1982). Mt. Erebus has a variety of different geothermal features, including ice chimneys (Panter & Winter, 2008), subglacial caves (Tebo et al., 2015), and large areas of exposed hot soils maintained by active steaming fumaroles (T. Ilanko et al., 2019). The geochemistry of these individual geothermal features on Mt. Erebus is unique and highly varied (R. Esser et al., 2004; T. Ilanko et al., 2019). There are two large geothermal sites on Mt. Erebus, Tramway Ridge and Western Crater, that contain multiple geothermal source points.

Tramway Ridge is the largest terrestrial geothermal feature on Mt. Erebus, characterised by both active (steam emitted) and passive (no steam emitted) fumaroles (Herbold, Lee, et al., 2014). Previous research in Tramway has shown that the year-round ground temperature averages 65 °C next to the active fumarole and supports a complex ecosystem, including thick cyanobacterial mats, moss, lichen, mites, and even nematodes (*Management Plan for Antarctic Specially Protected Area (ASPA) No. 130*, n.d.). In a preliminary study of microbial life at Tramway Ridge, a DNA fingerprinting method (ARISA) was used to determine the key drivers of microbial communities within one short temperature gradient (Soo et al., 2009). This study concluded that microbial diversity quickly increases away from the fumarole driven by temperature (Soo et al., 2009). A second follow up study examined a depth profile in soils taken directly adjacent to the fumarole at 65 °C (Herbold, Lee, et al., 2014; Soo et al., 2009) and showed that the surface community was dominated by cosmopolitan taxa while those deeper in the soil appeared endemic. These preliminary studies have shown that temperature and pH appear to drive microbial communities' composition and structure at Tramway Ridge (Herbold, Lee, et al., 2014; Soo et al., 2009). However, the actual geochemical factors that drive the structure of microbial communities between sites on Mt. Erebus are not adequately understood.

This study examined soil microbial communities across three steep physico-chemical gradients at two disparate sites, Tramway Ridge (TR1: -77.519722, 168.960556 DD; TR2: -77.519444, 169.074722 DD) and Western Crater (WC: -77.519167, 167.187778 DD), on Mt. Erebus, Antarctica. The two transects at Tramway Ridge allowed for the examination of within-site variability. The transect at Western Crater provided the first analysis of a passive fumarole geothermal site on Mt. Erebus. It is hypothesised that the unique physico-chemical features at each site drive microbial community composition and structure. These gradients were examined using DNA metabarcoding and a variety of physico-chemical analyses (pH, conductivity, total carbon, total nitrogen, ICP-MS elemental analysis, gravimetric water content) to assess the impact of temperature and geochemistry on soil microbial communities from geothermal sites on Mt. Erebus.

2.2 Methods

2.2.1 Site Description and Soil Collection

Soil samples were collected from two sites on Mt. Erebus, Antarctica, in November 2019. These included two temperature gradients on Tramway Ridge (TR1: -77.519722, 168.960556 DD; TR2: -77.519444, 169.074722 DD) and one transect in Western Crater (WC: -77.519167, 167.187778 DD) (**Figure 2.2-1**). Tramway Ridge is an Antarctic Specially Protected Area (ASPA) at an elevation of approximately 3,450 m.a.s.l on the northwest slope of Mt. Erebus. It is an 0.08032 km^2 area located at the terminus of lava flow with many active steaming fumaroles that support extensive moss beds and cyanobacterial mats (Harpel et al., 2004; *Management Plan for Antarctic Specially Protected Area (ASPA) No. 130*, n.d.; *Management Plan For Antarctic Specially Protected Area No. 175*, 2014). Western Crater is a much smaller passive fumarole site located 1.5 km from Tramway on the southwestern side of the mountain at an elevation of approximately 3550 m.a.s.l There is no noticeable moss or established cyanobacterial mats at this site. Soil temperatures were measured *in situ* at both sites with a Checktemp 1C electronic temperature sensor (Hanna Instruments, Rhode Island, USA) at 10 °C intervals from the hottest site. The top centimetre of soil was removed, the next 2 cm depth collected using a sterile spatula and placed into a 50-ml sterile Falcon tube, and the sample frozen until later analysis.

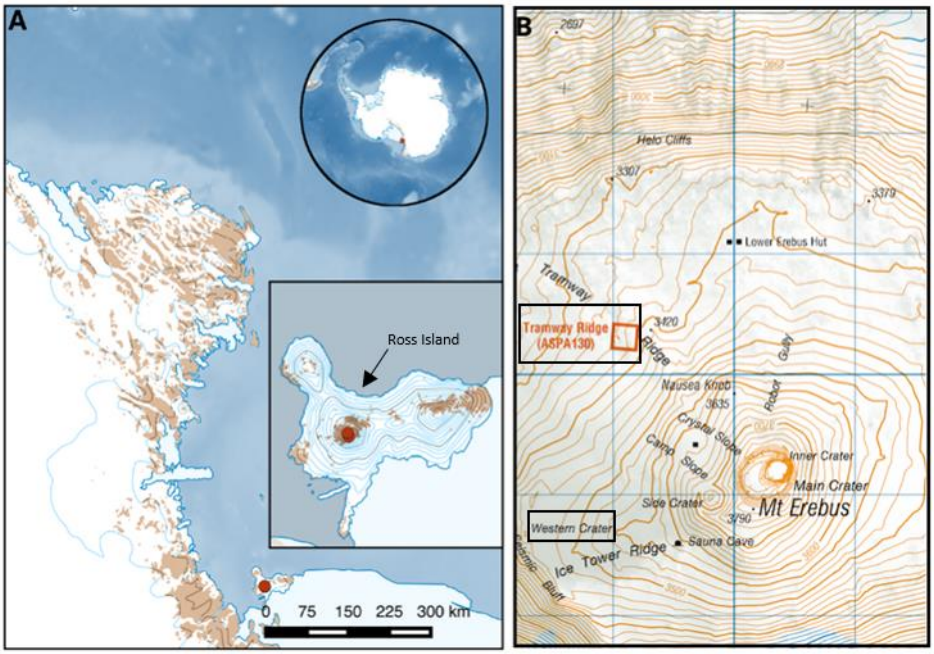

Figure 2.2-1: A) Map of Antarctica (circular inset) and Victoria Land and within Ross Island (rectangular inset) showing the location of Mt. Erebus. B) The sampling sites on Mt. Erebus. Source: QGIS, Quantarctica, 2018, Land Information New Zealand

2.2.2 Geochemistry

Geochemical analyses were performed to characterise all soil samples collected from the Tramway Ridge and Western Crater sites. The following analyses were conducted: Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) for elemental characterisation, pH, electrical conductivity (EC), gravimetric water content (GWC), total carbon (TC), and total nitrogen (TN). These analyses were all conducted at the University of Waikato (Hamilton, New Zealand) support facilities.

2.2.2.1 Elemental Composition

The following elements were analysed by ICP-MS: B, Na, Mg, P, K, Ca, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Ga, As, Se, Sr, Ag, Cd, Ba, Tl, Pb, and U. Briefly, 1g of each sample was ground using a Mixer Mill MM 400 (Retsch, Haan, Germany) at a frequency of 27.5 Hz for 1 min and 50 sec. The ground soil samples were digested with 1:6 dilution 10 mL HCl and 1:3 dilution 4 mL HNO_3 at 80 °C for 30 minutes. The samples were then diluted to 100 mL, filtered with a 0.45 µM filter to remove soil particles and diluted to an acid: water 1:5 concentration to further

reduce acid concentration. Ten millilitres from each sample was then acidified with 200 µL of pure Nitric acid before analysis. Elemental composition was measured by using an Agilent 8900 ICP-MS (Agilent Technologies, Santa Clara, California, USA).

2.2.2.2 pH and Electrical Conductivity

pH and EC were measured by creating a slurry by adding soil to milliQ water at a ratio of 1:2.5 in 50 mL sterile Falcon tubes, according to Barrett et al. (2009). pH was measured using a HI2213 Basic pH / ORP / °C Meter / with 3-Point Calibration (Hanna Instruments, Rhode Island, USA). pH measurements were adjusted for temperature and calibrated using known pH standards. EC was measured using a Thermo Scientific Orion 4-Star Benchtop pH/Conductivity Meter using standards (Thermo Fisher Scientific) on the same slurry.

2.2.2.3 Total Carbon, Total Nitrogen, and Gravimetric Water Content (GWC)

Each soil sample was weighed (3 g) and placed in a drying oven at 105 °C until the weight was unchanged. The GWC was calculated as the percentage of weight loss to measure gravimetric water content (Barrett et al., 2009). For total carbon and total nitrogen measurements, the dried samples were ground on a Mixer Mill MM 400 (Retsch, Haan, Germany) at a frequency of 27.5 Hz for 1 min and 50 sec. Approximately 100 mg of each dried sample was then measured on a CHNOS elemental analyser (Elementar, Langenselbold, Germany) in the Stable Isotope Facility at the University of Waikato.

2.2.3 DNA Extraction

Genomic DNA was extracted from 0.5–0.9 g of soil from each sample using a CTAB/bead beating method adapted from Herbold et al. (2014) by extending the incubation time from 30 minutes to an hour. Where DNA yield was low, multiple (2–3) extractions were carried out, and the DNA pooled. Genomic DNA was quantified using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Massachusetts, USA).

2.2.4 DNA Amplification by PCR and Ion-Torrent Sequencing

The 16S rRNA gene (V4-V5 region) was amplified by PCR and using the Earth Microbiome primers, 515F and 926R (Parada et al., 2016; Quince et al., 2011), adapted as fusion primers for Ion Torrent sequencing by including a unique tag and IonXpress barcode to distinguish individual samples and enable Ion Torrent sequencing (Whiteley et al., 2012). The 20 µL reaction mixture included 0.24 mM dNTPs, 1.2x PCR buffer, 6 mM $MgCl_2$, 0.016 mg/ml BSA, 0.2 mM of each primer, 0.024 U Taq polymerase (Thermo Fisher Scientific, Massachusetts, USA), and 9 ng of genomic DNA, except for one sample (WC20) that contained 3 ng DNA due to low DNA yield. The following PCR protocol was followed: initial denaturation 94 °C for 3 min followed by 30 cycles for 94 °C for 45 sec, 50 °C for 1 min, 72 °C for 1.5 min, and final extension of 72 °C for 10 min. All PCR reactions were run on an Applied Biosystems ProFlex PCR System (Thermo Fisher Scientific). PCR reactions were run in triplicate to account for possible PCR bias. PCR products were run on a 1 % agarose gel with SybrSafe for 25 mins at 70V and then visualised using an Alpha Innotech Imaging System (Alpha Intech). The triplicate PCR products from each sample were pooled, and 25 µL of each was treated with Invitrogen SequalPrep Normalization (Thermo Fisher Scientific) to purify, normalise the PCR product concentration, and remove DNA fragments smaller than 100 bp. To construct the Ion Torrent amplicon library, 2µL from each purified PCR product was pooled together. This library was quantified via Qubit 2.0 Fluorometer (Thermo Fisher Scientific), and the number of amplicons determined in order to not overload the sequencing plate and the Library moved through the Ion PGM™ Template IA 500 preparation Kit, as per manufacturer instructions (Thermo Fisher Scientific). Ion PGM™ Hi-Q™ View Sequencing Kit (Thermo Fisher Scientific) was used to prepare the library for sequencing. The library was then added to an Ion 318™ Chip Kit v2 BC (Thermo Fisher Scientific). Samples were sequenced on an Ion Torrent PGM (Thermo Fisher Scientific) at the University of Waikato DNA Sequencing Facility.

2.2.5 Sequence Quality Control and Taxonomic Assignment

The raw sequences received from the Ion Torrent PGM (Thermo Fisher Scientific, Massachusetts, USA) were processed using the DADA2 pipeline according to a previously published workflow (Benjamin J. Callahan et al., 2016). Sample filtering, trimming, error rates learning, dereplication, and ASV inference were performed with default settings and additional parameters recommended for Ion Torrent (HOMOPOLYMER_GAP_PENALTY=-1,

BAND_SIZE=32). Chimeras were removed with the removeBimeraDenovo function using the method "consensus". Taxonomy was assigned with the function assignSpecies using the native implementation of the naive Bayesian classifier and a DADA2-formatted reference database using the Silva database version 138 (10.5281/zenodo.3986799).

Taxonomic filtering was performed by removing eukaryotic, mitochondrial, and chloroplast sequences assignments. An unrooted phylogenetic tree was built with the neighbour-joining method, maximising the likelihood with a gamma model distribution, using the decipher (Wright, 2016) and phangorn (Schliep, 2011) packages in R according to the workflow provided by (B J Callahan et al., 2016).

2.2.6 Statistical Analysis

All statistical analyses were conducted in R (version 4.0.1) using base R functions unless otherwise stated (R Core Team, 2020). Plots were obtained using package ggplot2 (Wickham et al., 2020), and data wrangling was performed with tidyverse (Wickham et al., 2020).

A Kruskal-Wallis test was performed to compare the response of physico-chemical factors between different sampling site locations and along individual transects. Whenever a difference was found, a Wilcoxon rank-sum test, using a Benjamini-Hochberg correction for multiple testing, was employed to test for pairwise differences. Microbial community structure was assessed with a principal coordinates analysis (PCoA) on a UNIFRAC distance matrix using Phyloseq (McMurdie & Holmes, 2013). Statistical differences were evaluated with a permutational analysis of variance (PERMANOVA) using the R package vegan (Oksanen et al., 2019).

A Mantel test with 999 permutations was employed to correlate physico-chemical factors with the microbial community structure. Whenever a significant difference was found ($p < 0.05$), the physico-chemical factor was selected as a driver of the biological data to inspect how these significant factors would influence the biological data using a distance-based Redundancy Analysis (dbRDA) that was based on z-score normalisation of the physico-chemical data (Legendre & Anderson, 1999). These analyses were performed with the functions implemented in the R package vegan.

Alpha diversity was assessed with the Shannon index using the R package vegan and a Venn diagram using the R package VennDiagram (Chen, 2016) to portray the unique ASVs in each transect. Random Forests modelling employing the R package randomforest (Liaw & Wiener, 2002) based on 1,000 decision trees was used to predict the importance of each ASV in each Tramway Ridge transect and between the Tramway Ridge and Western Crater sites.

The results from the 16S amplicon sequencing will be publicly available on GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

2.3 Results

2.3.1 Soil Geochemistry Characterisation

Soil temperatures were measured *in situ* and ranged from 10–64 °C (**Table 2.3-1**) across all three transects. The two transects at Tramway Ridge had temperatures ranging from 24–64 °C, and at Western Crater, the temperature ranged 10–50 °C. The transects at Tramway Ridge and Western Crater showed diverging physico-chemical features. Western Crater generally had a higher pH (6.93–9.20) than Tramway Ridge (pH 4.04–6.20). Meanwhile, the electrical conductivity (EC), gravimetric water content (GWC), total carbon (TC), and total nitrogen (TN) showed higher values at Tramway Ridge than at Western Crater. EC ranged from 183.4–957 µS/cm, GWC from 9.5–20.7 %, TC from 0.34–2.9 %, and TN from 0.06–0.46 %, whereas for Western Crater, EC ranged from 4.26–6.39 µS/cm, GWC from 6.4–11.9 %, TC from 0.08–1.16 %, and TN from 0.02–0.21 % (**Table 2.3-1**).

At WC, pH increased with temperature, while TC, TN, and EC showed the highest value at the highest temperature sample (WC50). For the transects in Tramway Ridge, there was no observed correlation between temperature and any other physico-chemical parameter. Interestingly, there was a disparity in physico-chemistry between the two Tramway Ridge transects. In TR1, EC and GWC generally increased as temperature increased, while the opposite was observed in TR2, as EC and GWC generally decreased with increasing temperature. The C:N ratio increased with temperature at TR2, but at TR1, there was no clear trend.

Table 2.3-1:The temperature at the sampling site at each transect

Tramway Ridge Transect 1 (TR1)	Tramway Ridge Transect 2 (TR2)	Western Crater Transect (WC)
62 °C	64 °C	50 °C
52 °C	44 °C	40 °C
42 °C	34 °C	35 °C
32 °C	24 °C	30 °C
		20 °C
		10 °C

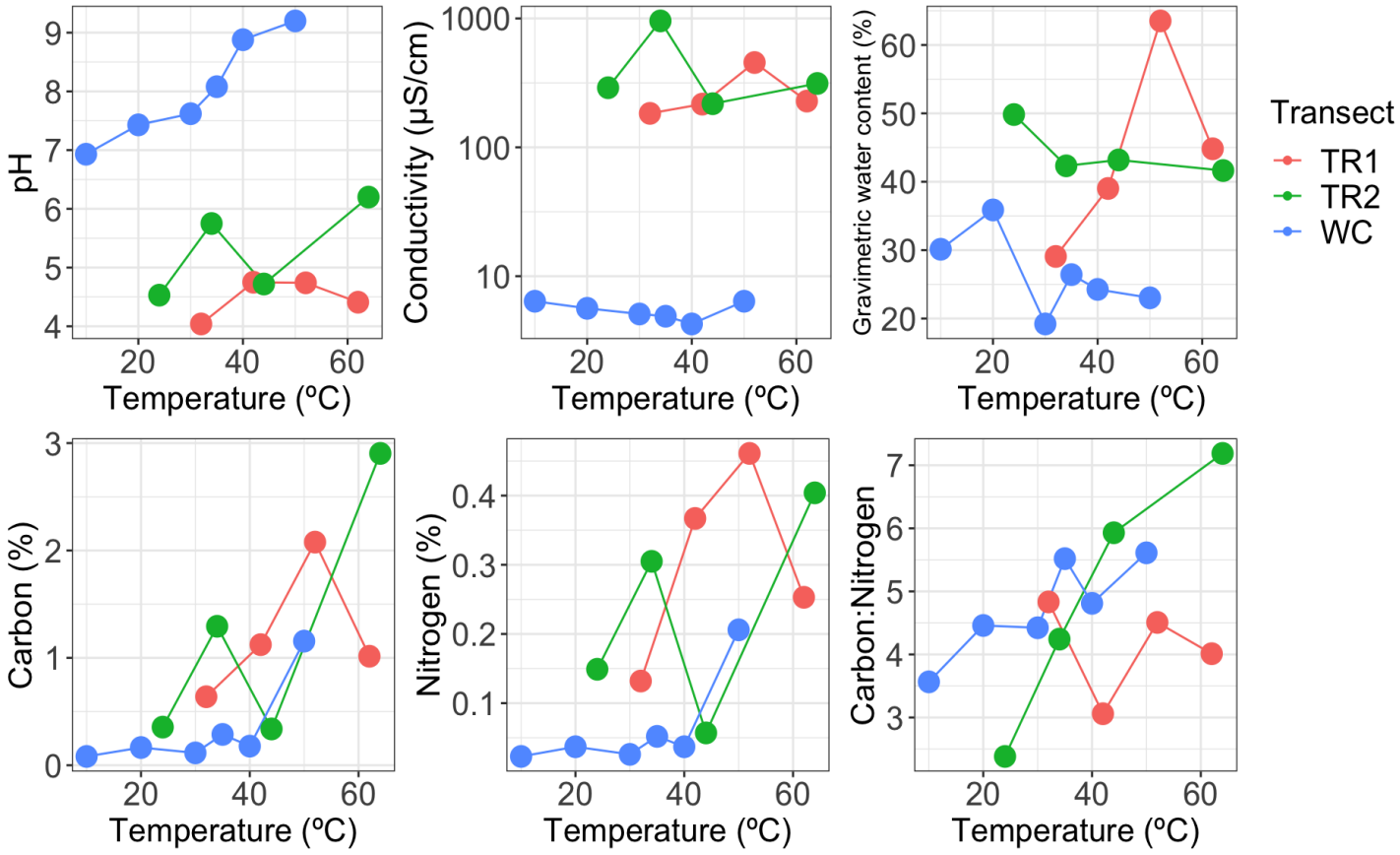

Figure 2.3-1: **Environmental parameters and nutrients for each transect**. The temperature was the chosen parameter to define the sampling sites and was used as a proxy for distance from the fumarole

A Kruskal-Wallis test showed that pH was significantly different at WC and TR (p-value = 0.006), and a Wilcoxon test showed that pH was significantly higher at WC, followed by TR2 and TR1 (p-value < 0.05). In addition, WC exhibited significantly lower GWC and EC values than TR1 and TR2, (p-value = 0.02) and (p-value = 0.006) respectively. A Wilcoxon test showed that GWC and EC were significantly different between WC and both TR1 and TR2 (p-value < 0.05), but no difference could be seen between TR transects

ICP-MS was used to analyse the concentration of 33 elements in each soil sample. The results can be seen in Appendix A and in **Figure 2.3-2**, where elements of interest have been highlighted. WC exhibited similar concentrations of Cu, Fe, Pb, S, V, and Zn across the entire transect, except at the highest temperature site (WC 50), where all these elements were at least three times higher than at any other site (**Figure 2.3-2**). In contrast, at TR1, five elements (Cu, Fe, Ni, S and Zn) showed their highest concentrations at the coldest sampling site TR1 32. At this sampling site, these elements showed a concentration three times higher than at the next sampling site, TR1 42. The concentrations then increased gradually to the hottest sampling site (TR1 62) but never reaching the values seen at TR1 32. The exception to this trend was sulphur, which showed similar concentrations at TR1 32 and TR1 62 (**Figure 2.3-2**), unlike the other elements that still exhibited concentrations approximately two times lower than at TR1 32. Interestingly, the TR2 transect showed different trends for these elements, with Cu, Fe, Ni, S and Zn exhibiting a conspicuously lower concentration at TR2 44, the sampling site with the second-highest temperature in the transect (approximately three times less than at the remaining three sampling sites in the transect). It was interesting to note that TR1 42 also exhibited the lowest concentrations for these same elements, although the actual concentrations for Cu, Fe, Ni, S and V were two times higher than at TR2 44 (**Figure 2.3-2**).

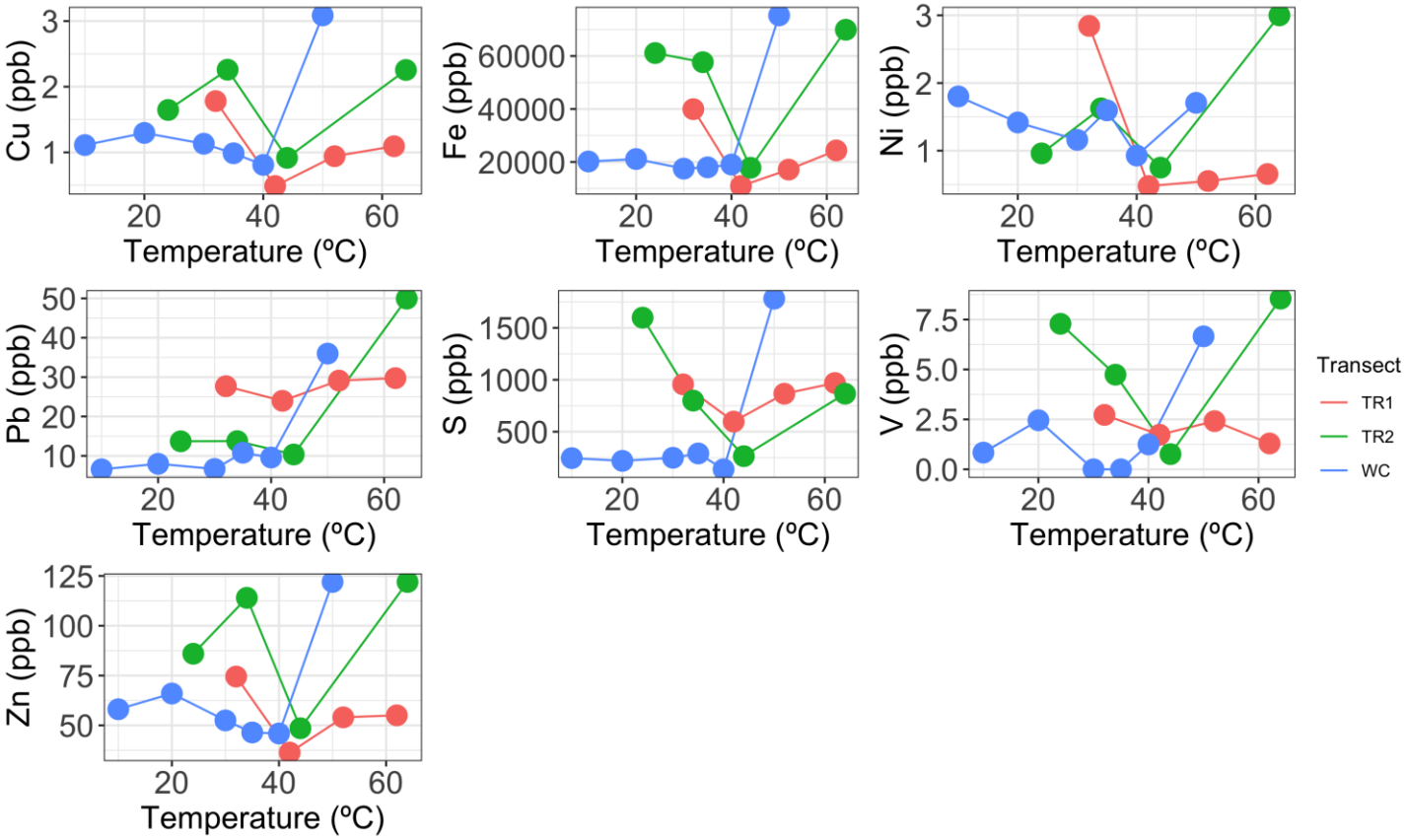

Figure 2.3-2: Elemental concentration (ppb) in the soil at each sampling site.

2.3.2 DNA Concentration in the Soils

DNA concentration was used as a proxy for microbial biomass. Tramway Ridge samples had higher DNA yield ranging from 0.02–3.3 µg/g compared to Western Crater with 0.005–0.164 µg/g (**Table 2.3-2**). DNA yield improved for Western Crater samples when the ratio of reagents to soil was increased, and amplification was successful with dilution that minimise contaminants' interference. The extracted DNA concentration showed no increase with increasing temperature or any other physico-chemical parameter measured.

Table 2.3-2: Amount of DNA (µg) per gram of soil (ww) extracted from each sample.

Tramway Ridge		Western Crater	
Temperature	**DNA concentration (µg/g)**	**Temperature**	**DNA concentration (µg/g)**
TR2 64 °C	3.30	WC 50 °C	0.053
TR1 62 °C	1.87	WC 40 °C	0.009
TR1 52 °C	2.68	WC 35 °C	0.146
TR2 44 °C	2.91	WC 30 °C	0.164
TR1 42 °C	2.66	WC 20 °C	0.005
TR2 34 °C	3.34	WC 10 °C	0.049
TR1 32 °C	2.52		
TR2 24 °C	0.02		

2.3.3 Sequencing Data Quality Control

PCR amplicon sequencing of the 16S rRNA gene showed a varied read distribution after quality filtering. At TR1, the number of reads ranged from 34069 and 2803 per sample, with a median value of 24342; at TR2, the number of reads ranged from 30992 and 4476 reads per sample, with a median value of 30708, and at WC, the number of reads ranged from 32815 and 3086 reads per sample, with a median value of 24941 (**Table 2.3-3**).

Table 2.3-3: Number of sequences before (reads in) and after filtering, ASV inference and chimaera detection (reads out).

Tramway Ridge				Western Crater			
Temperature	**Reads In**	**Reads Out**	**Percentage remained**	**Temperature**	**Reads In**	**Reads Out**	**Percentage remained**
TR2 64 °C	85245	30557	36 %	WC 50 °C	54799	26677	49 %
TR1 62 °C	82757	30542	37 %	WC 40 °C	17927	8232	46 %
TR1 52 °C	40955	2803	7.0%	WC 35 °C	85737	32815	38 %
TR2 44 °C	73412	30557	42 %	WC 30 °C	70644	28693	41 %
TR1 42 °C	39804	30542	46 %	WC 20 °C	6401	3086	48 %
TR2 34 °C	45593	4476	10 %	WC 10 °C	60093	23205	39 %
TR1 32 °C	69373	34069	49 %				
TR2 24 °C	73326	30859	42 %				

For further read quality control, a Principal Coordinates Analysis (PCoA) based on UniFrac distance was employed to compare the entire dataset with the dataset trimmed for samples with a lower number of reads (< 10,000). When examining all 14 sampling sites (3 transects), the PCoA explained ca. 46 % of the variation between samples. When the samples with a low number of reads were removed, the PCoA explained ca. 51 % of the variation (Appendix B). The gain in explained variation was therefore marginal (5 %). Given the small number of samples (14 samples in total), all the sampling sites were retained for subsequent analysis.

2.3.4 Taxonomic Diversity

A total of 940 Archaea and Bacteria ASVs were identified across all samples from both sites. WC had the highest number of ASVs (595), followed by TR2 (355) and TR1 (298) (**Figure 2.3-3**). There were a total of 496 unique ASVs at WC, 165 unique ASVs at TR2, and 109 ASVs unique to TR1. TR1 and TR2 shared 106 ASVs, while WC only shared six ASVs with TR2 and 15 ASVs with TR1 (**Figure 2.3-3**). All three sites shared 43 ASVs which corresponded to ca. 5 % of the total number of ASVs present across all transects (**Figure 2.3-3**). As expected, the

TR1 and TR2 had considerably more ASVs in common than with WC, potentially reflecting the physico-chemical differences between the sites.

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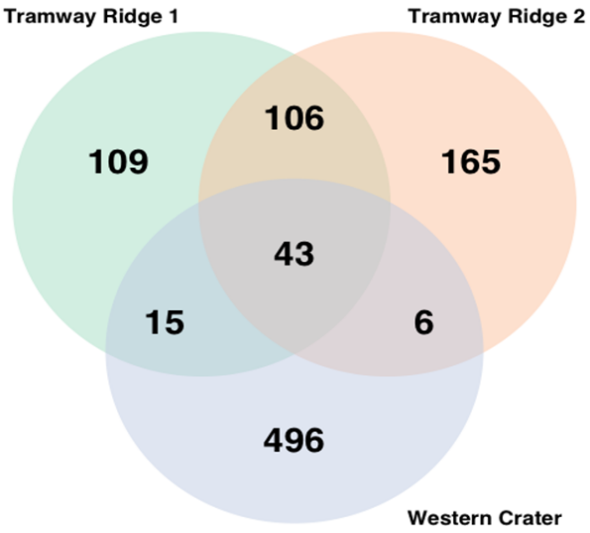


Figure 2.3-3: All unique and shared ASVs on the three analysed transects

As the difference between the ASVs was so drastic between Tramway Ridge and Western Crater, a random forest analysis was employed to classify samples by site. The confusion matrix showed the random forest analysis could correctly classify the samples as Western Crater or Tramway Ridge, with 100 % accuracy (**Figure 2.3-4A**). Random forest was 75 % accurate (**Figure 2.3-4B**) when distinguishing between the two Tramway Ridge transects, indicating the inability to clearly differentiate between TR1 and TR2. These results support the suggested similarities between TR1 and TR2 and the distinctiveness of WC, as shown in **Figure 2.3-3**.

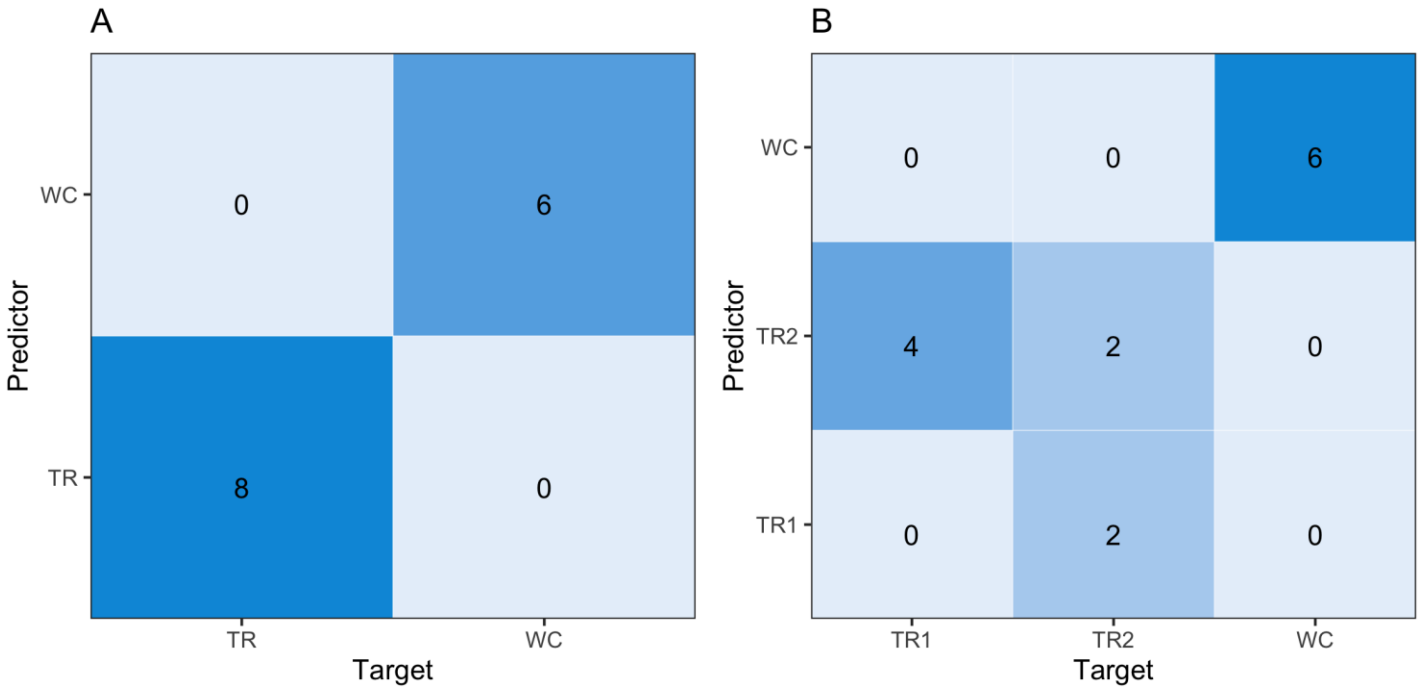


Figure 2.3-4: Confusion matrix for Random Forest data comparing both A) Site B) Transect

Examination of microbial diversity showed considerable variation within and between sites even at the Domain level. Tramway Ridge samples had a higher relative abundance of Archaea ranging from 13–57 %, with the highest relative abundance seen at TR1 32 (**Figure 2.3-5A**). Comparatively, WC was dominated by Bacteria (93–99.5%), but Archaea were still present at all sampling sites, with the highest relative abundance found at WC 50 (7%). Proteobacteria and Chloroflexi were the only phyla present at every sampling site in all transects, and, interestingly, both presented the highest relative abundance at WC 20 (**Figure 2.3-5B**). Acidobacteriota were present in every sampling site at WC, associated with the mostly alkaline pH. Acidobacteriota also had a higher relative abundance at WC compared to TR1 and TR2. Conversely, Crenarchaeota was present at all temperatures in the Tramway Ridge transects and showed a significantly higher relative abundance than at any of the WC sites. Cyanobacteria and Deinococcota were present in all three transects. However, they exhibited a noticeable increase in relative abundance in samples collected at or above 50 °C.

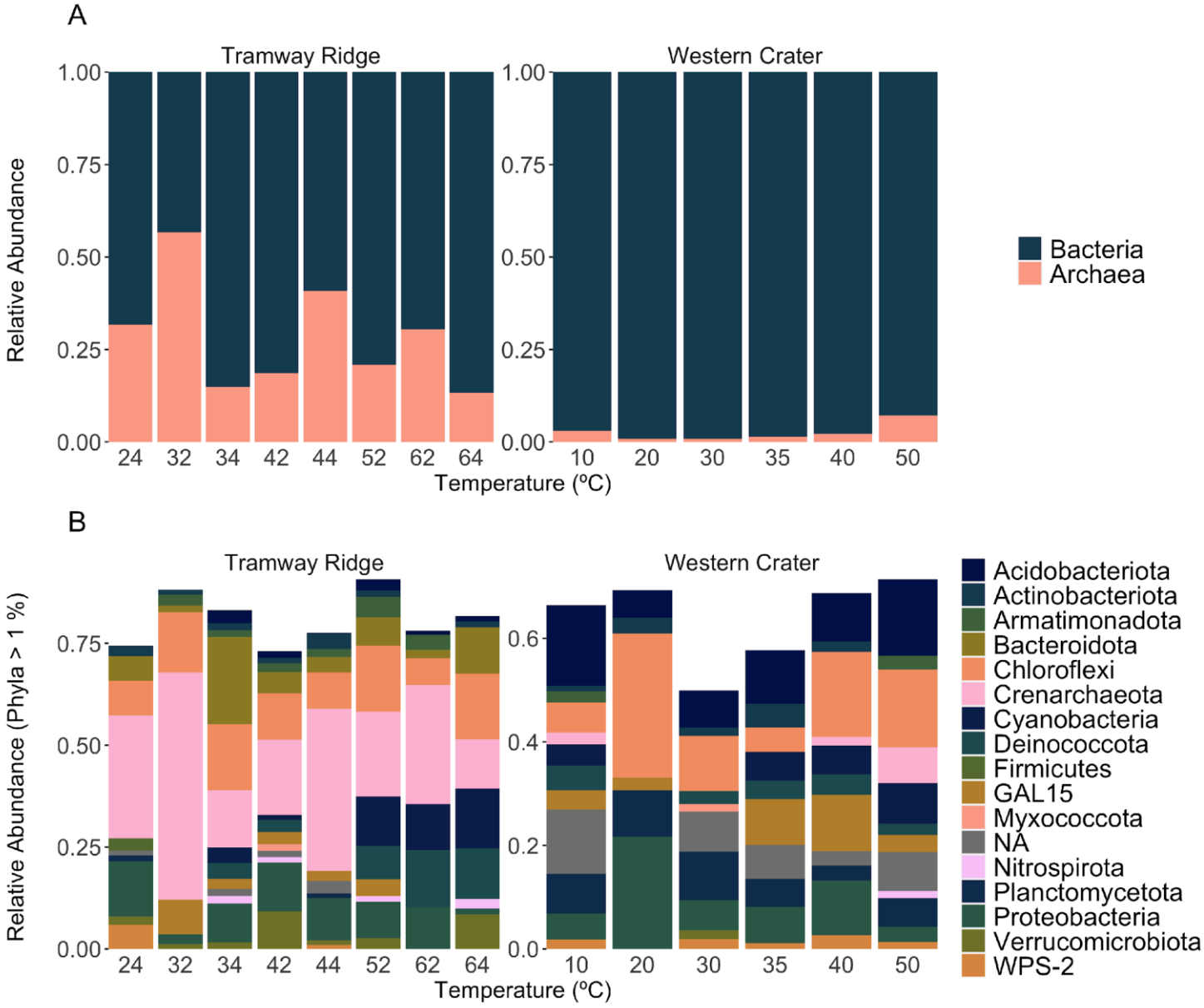


Figure 2.3-5: Relative abundance of the domain and top phyla (> 1 %) level. A) Domain relative abundance and B) Phyla relative abundance.

To further investigate the taxonomic diversity at Tramway Ridge and Western Crater, a comparative analysis of ASVs with the highest relative abundances (Bacteria > 5 % and Archaea > 1 %) was performed (**Figure 2.3-6**). The ASVs varied considerably across all transects. However, all three transects shared seven ASVs in common. Those ASVs common to all three transects included ASV 1, Nitrososphaeria, phylum Crenarchaeota, which was the only

archaeon present with high relative abundance at all three transects and at every sampling site except WC 20. The remaining six ASVs present in all three transects were Bacteria. ASV 3 was assigned to the class Cyanobacteriia of the phylum Cyanobacteria. ASV 4 could not be classified beyond the domain Bacteria. ASV 6 was assigned to the phylum GAL15 and could not be classified any further. ASV 7 was assigned to the class AD3 of the Chloroflexi phylum. ASV 9 was assigned to the class Anaerolinae of the phylum Chloroflexi. Finally, ASV 20 was assigned to the class Acidobacteriea of the phylum Acidobacteriota.

The distribution of Archaea across transects showed that the ASVs could be assigned to phylum Crenarchaeota. The Tramway Ridge transects included ASV 14 assigned to the order SCGC AB-179-E04; ASV 15 and ASV 19 assigned to the genus *Candidatus Nitroscomicus*; and ASV 27 assigned to the genus *Candidatus Nitrososphaeara*. ASV 65, assigned to the genus *Candidatus Nitrososphaera*, was the only archaeal ASV found only at the Western Crater transect. The distribution of Archaea across transects might have been driven by differences in taxonomy, as *Candidatus Nitroscomicus* appears to be exclusively at Tramway Ridge, whereas *Candidatus Nitrosasphaera* seems to occur both at Tramway Ridge and Western Crater.

The Tramway Ridge transects had similar composition and richness of Bacteria. The five most abundant ASVs included ASV 2, assigned to the genus *Mizugakiibacter* (phylum Proteobacteria); ASV 5, assigned to the genus *Meiothermus* (phylum Deinococcota); ASV 8, assigned to the genus *Candidatus Udaeobacter* (phylum Verrucomicrobiota); ASV 11, assigned to the family *BSV26* (phylum Bacteroidota); ASV 18, assigned to the family *Chitinophageae* (phylum Bacteriodota); And finally, ASV 41, assigned to the phylum WPS-2. This shows a significant overlap between the ASVs present at both TR1 and TR2, as seen in the confusion matrix (**Figure 2.3-4**).

The Western Crater microbial community was different from both Tramway Ridge transects in both richness and composition. Six most abundant Bacteria ASVs were present at all temperatures in Western Crater: ASV 16, assigned to the GAL15 phylum; ASV 17, assigned to the genus *Candidatus Koribacter* (phylum Acidobacteriota); ASV 57, assigned to the class AD3 (phylum Chloroflexi); ASV 60, assigned to the class Methylovirgula (phylum

proteobacteria); ASV 107, assigned to the class AD3 (phylum Chloroflexi); And finally, ASV 164, assigned to the genus *Methylocapsa* (phylum Proteobacteria).

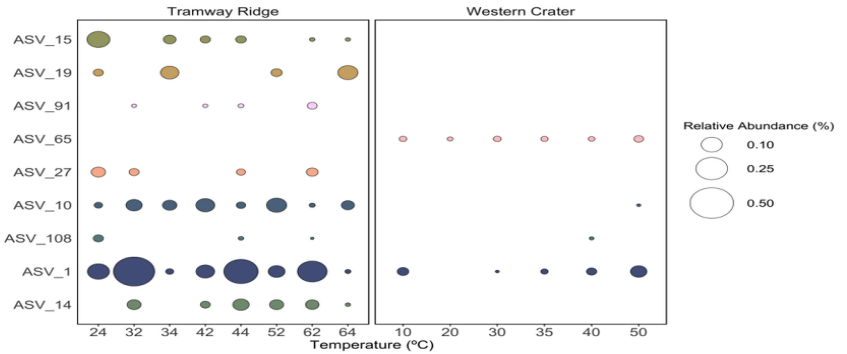
The taxonomy of the ASVs obtained in this study was further examined by blasting the sequence against the NCBI 16S rRNA database. Four ASVs in this data set showed 100 % identity to sequences that had been previously reported for Tramway Ridge (Herbold et al. 2014). Two ASVs, ASV 4 and ASV 25, assigned to the genus *Meiothermus*, (phylum deinococcota). ASV 36, assigned to genus *Fischerella PCC-9339* (phylum Cyanobacteria), and ASV 61, assigned to genus *Pyrinomonas* (phylum Acidobacteriodota). Of all these, ASV 61 was the only ASV present at both Tramway Ridge and Western Crater, with the remaining ASVs exclusively found at Tramway Ridge. Additionally, ASV 47, assigned to genus *Anaerolinae*, showed 100 % identity to a sequence also reported in a previous study (Soo et al., 2009) of Tramway Ridge (Appendix C).

A

Phylum	Class	Order	Family	Genus	ASV
Crenarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Candidatus Nitrocosmicus	ASV_15
Crenarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Candidatus Nitrocosmicus	ASV_19
Crenarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Candidatus Nitrososphaera	ASV_91
Crenarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Candidatus Nitrososphaera	ASV_65
Crenarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Candidatus Nitrososphaera	ASV_27
Crenarchaeota	Nitrososphaeria	Nitrososphaerales	Nitrososphaeraceae	Candidatus Nitrososphaera	ASV_10
Crenarchaeota	Nitrososphaeria	Nitrosotaleales	Nitrosotaleaceae	unassigned	ASV_108
Crenarchaeota	Nitrososphaeria	SCGC AB-179-E04	unassigned	unassigned	ASV_1
Crenarchaeota	Nitrososphaeria	SCGC AB-179-E04	unassigned	unassigned	ASV_14

B

Phylum	Class	Order	Family	Genus	ASV
Acidobacteriota	Acidobacteriae	Subgroup 2	unassigned	unassigned	ASV_20
Acidobacteriota	Acidobacteriae	Acidobacteriales	Koribacteraceae	Candidatus Koribacter	ASV_17
Bacteroidota	Kryptonia	Kryptoniales	BSV26	unassigned	ASV_11
Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	unassigned	ASV_18
Chloroflexi	Anaerolineae	unassigned	unassigned	unassigned	ASV_9
Chloroflexi	AD3	unassigned	unassigned	unassigned	ASV_107
Chloroflexi	AD3	unassigned	unassigned	unassigned	ASV_57
Chloroflexi	AD3	unassigned	unassigned	unassigned	ASV_7
Cyanobacteria	Cyanobacteriia	Leptolyngbyales	Leptolyngbyaceae	Leptolyngbya PCC-6306	ASV_3
Deinococcota	Deinococci	Thermales	Thermaceae	Meiothermus	ASV_5
GAL15	unassigned	unassigned	unassigned	unassigned	ASV_16
GAL15	unassigned	unassigned	unassigned	unassigned	ASV_6
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylocapsa	ASV_164
Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylovirgula	ASV_60
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	Mizugakiibacter	ASV_2
unassigned	unassigned	unassigned	unassigned	unassigned	ASV_4
Verrucomicrobiota	Verrucomicrobiae	Chthoniobacterales	Chthoniobacteraceae	Candidatus Udaeobacter	ASV_8
WPS-2	unassigned	unassigned	unassigned	unassigned	ASV_41



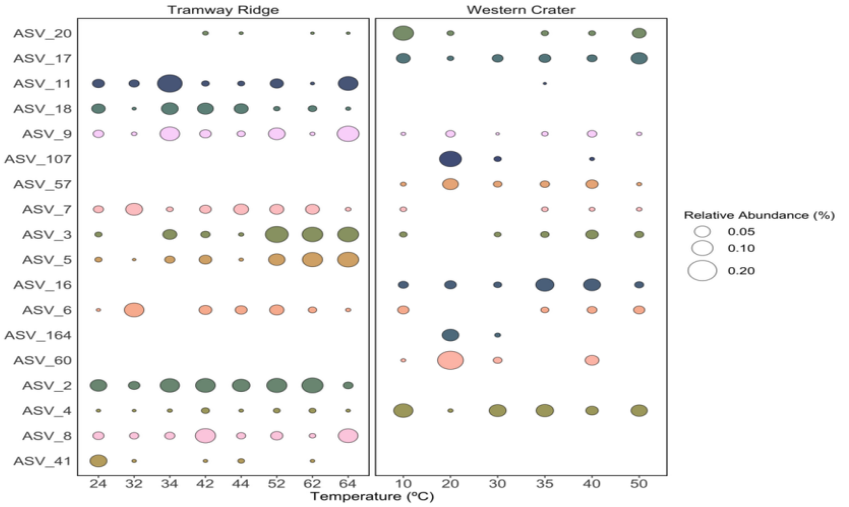


Figure 2.3-6: Classification of the Most Abundant ASV A) Archaea (> 1 %) B) Bacteria (> 5 %) The taxonomic assessment as assigned by SILVA. The size of the circle indicates relative abundance.

The random forest analysis was used to identify key ASVs that differentiate between samples at Tramway Ridge and Western Crater. The distribution and relative abundance of these key ASV were used to establish a relationship between the microbial communities at each site. The random forest analysis identified the ASVs that discriminated between sites, most of which occurred exclusively at only one site, with the majority of these ASVs found only at Western Crater. At Tramway Ridge, six most abundant ASVs, five Bacteria and one Archaea were identified as exclusive and driving the differences between Tramway Ridge and Western Crater (Appendix C). For Western Crater, fourteen most abundant ASVs, thirteen Bacteria and one Archaea were identified by the random forest as driving the differences between both sites (Appendix C).

2.3.5 Influence of pH and Temperature on the ASVs occurrence at each site

Samples were binned into groups with the same range of pH and temperature across both sites. Three pH groups were defined: greater than four and less than or equal to five (Tramway Ridge; six samples), greater than five and less than or equal to eight (all transects; five samples), or greater than eight and less than or equal to 10 (Western Crater; three samples). Three temperature groups were also defined: greater than 30 and less than or equal to 40 °C (all transects; four samples), greater than 40 and less than or equal to 50 °C (all transects; three samples), greater than 50 and less than or equal to 70 °C (Tramway Ridge; four samples). A Venn Diagram was employed to analyse the common ASVs within the defined groups (**Figure 2.3-7**) in order to visualise the relationship between ASVs and temperature and pH. For the temperature groups, 12 % of the ASVs were common to all the temperature ranges, similarly for the pH groups, 6.2 % of the ASVs were in common. In addition, there were eight ASVs in common between the highest and lowest pH groups and 15 ASVs in common between the highest and lowest temperature groups. This analysis identified that there were more distinct ASVs in different soil pH compared to different soil temperatures.

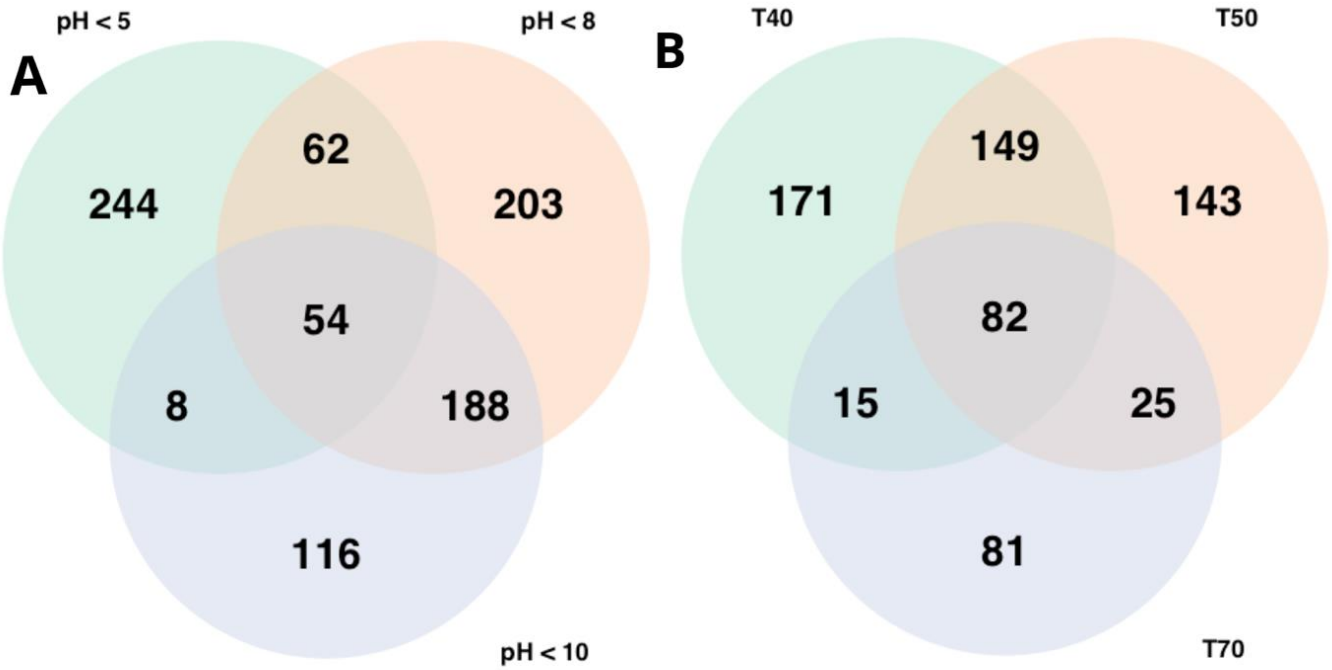


Figure 2.3-7: The separation of ASVs by A) pH and B) Temperature

2.3.6 Alpha Diversity

The alpha diversity of each sample was analysed using the Shannon index. The values ranged from 2.5–5.0 across all samples (**Figure 2.3-8**). The Shannon diversity index showed the highest richness and diversity at Western Crater. pH was chosen to visualise the alpha diversity results due to its utility in discriminating groups encompassing different ASVs, as seen in **Figure 2.3-7**. More alkaline samples had higher Shannon values, suggesting why Western Crater supported a more diverse microbial community. Overall, it appears that sampling sites with a higher pH had higher alpha diversity. However, within the individual transects, no alpha diversity trends were identified. Notably, the diversity of sample TR1 32 was very low, which was unsurprising given the high relative abundance of ASV 1 (**Figure 2.3-6**).

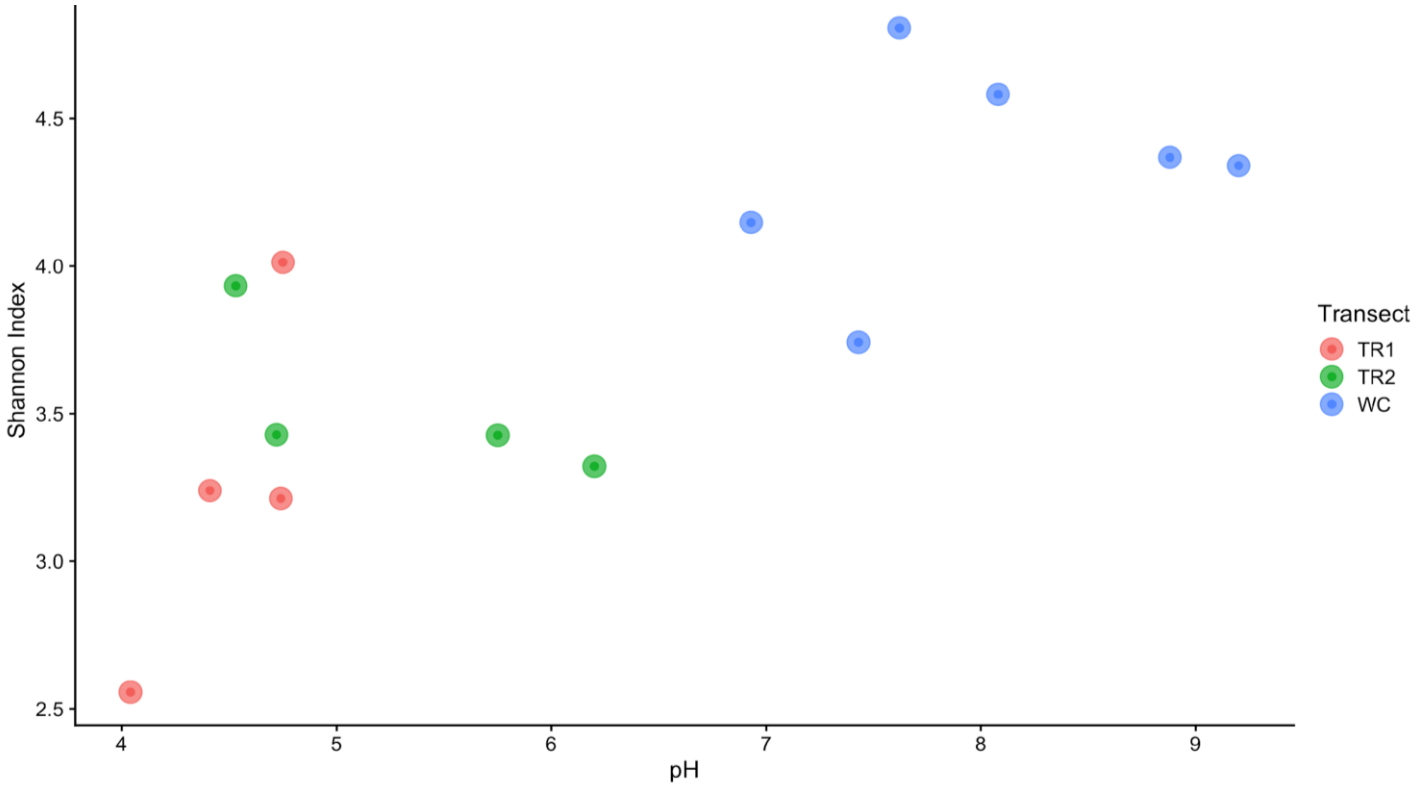


Figure 2.3-8: Shannon diversity for each of the samples presented by the pH of the sample. (TR1= Tramway Ridge transect one TR2 = Tramway Ridge transect two WC = Western Crater transect)

2.3.7 Beta Diversity

A distance-based Redundancy Analysis (dbRDA) based on the UniFrac distance of the biological data set and the Euclidean distance of the environmental data set was used to assess the degree of difference between the two sites and transects and what main abiotic factors were driving the variation in microbial community structure. This ordination showed Western Crater and Tramway Ridge to support clearly distinct communities (**Figure 2.3-9**). A PERMANOVA between sites showed that both Western Crater and Tramway Ridge were significantly different ($F = 5.8001$, p-value < 0.001), and a pairwise comparison showed that these differences were between Western Crater and both Tramway Ridge transects, however, between Tramway Ridge transects, no difference could be seen.

A Mantel Test, using Pearson product-moment correlation coefficient, was performed to discriminate the abiotic factors (p-value < 0.01) that could be driving the differences between the two sites. It was observed the TC, TN, EC, GWC, and to a lesser extent temperature, were selecting towards Tramway Ridge, whereas pH and P, which were higher in WC, were selecting towards Western Crater (**Figure 2.3-9**).

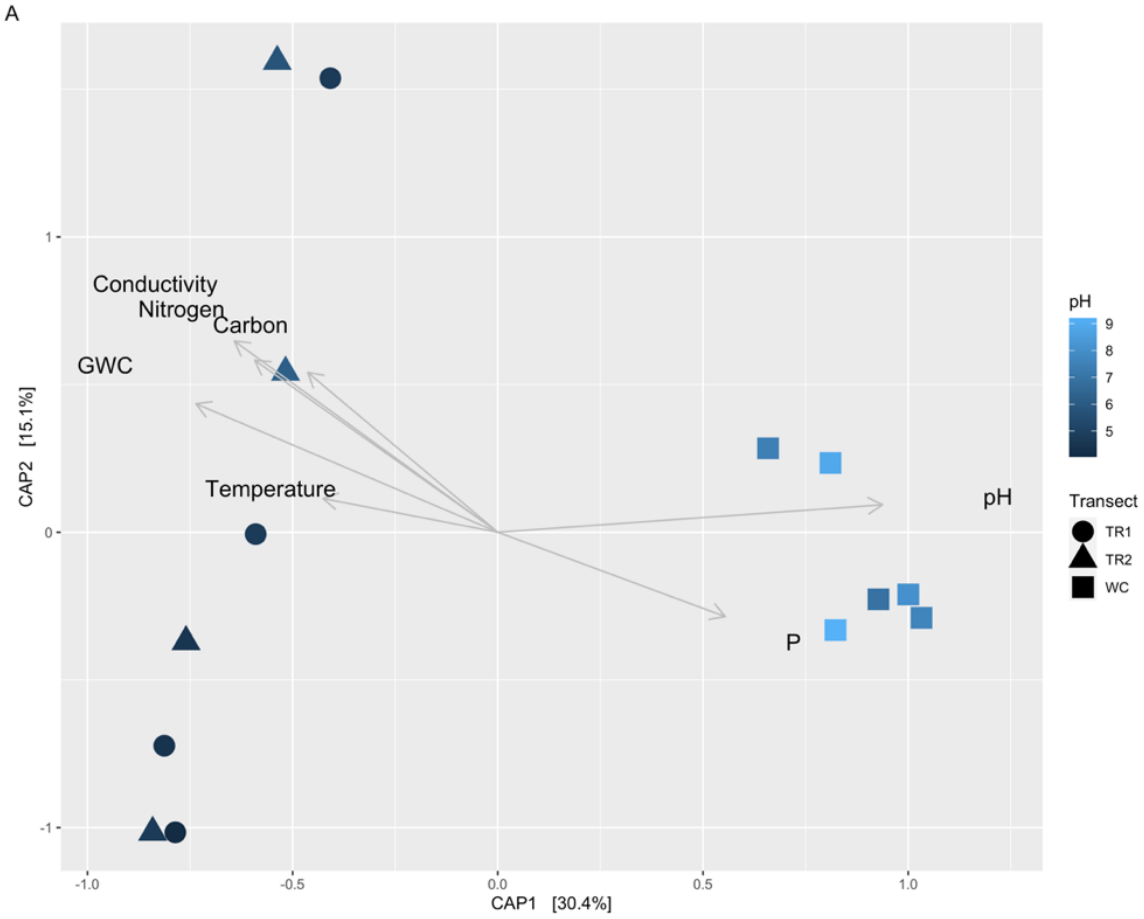


Figure 2.3-9: A distance-based Redundancy Analysis (dbRDA) of Tramway Ridge and Western Crater based on Bray-Curtis distance. It used to evaluate the effect of abiotic factors on microbial communities. The total variance is explained by each axis in brackets.

When each site was analysed separately with a PCoA, it was possible to see Tramway Ridge samples responding to pH along the PCoA first axis (**Figure 2.3-10A**), whereas for Western Crater, no pH driven response could be seen (**Figure 2.3-10B**). Moreover, grouping by temperature could not be seen for any site.

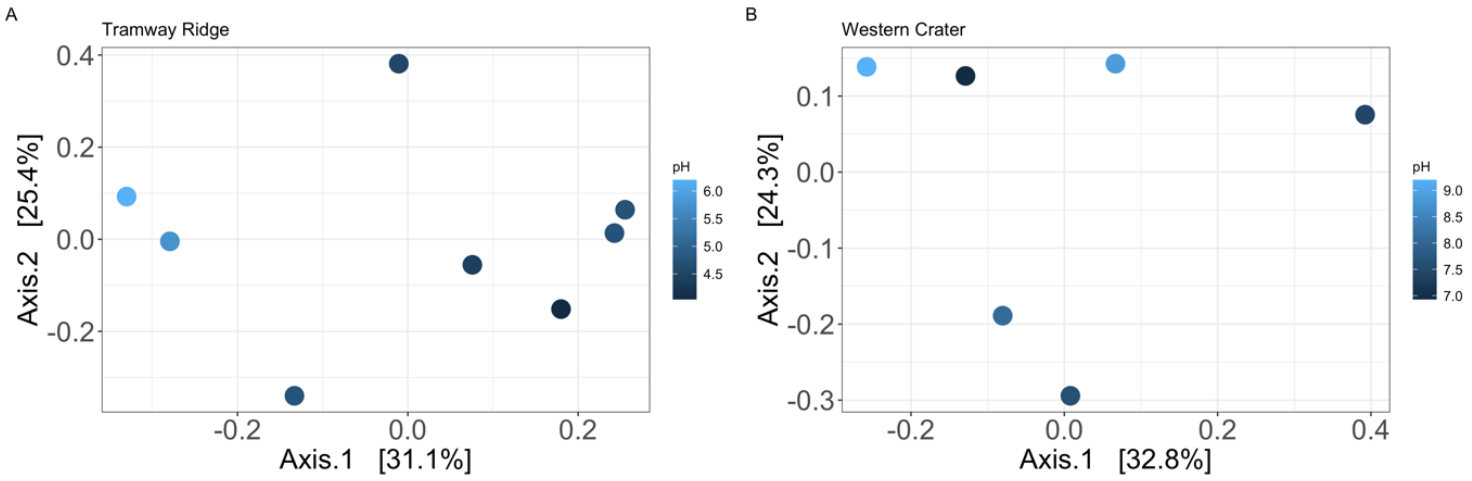


Figure 2.3-10: A Bray-Curtis distance PCoA of A) Tramway Ridge transects and B) **Western Crater.** The total variance is explained by each axis in brackets.

When Tramway Ridge samples were examined together with samples from transects from previous studies (Herbold et al., 2014; Soo et al., 2009), sampling sites could be seen on the PCoA grouping by both pH and temperature (**Figure 2.3-11**). There were three distinct groups: low temperature range (greater than 0 and less than or equal to 20 °C), medium temperature range (greater than 20 and less than or equal to 50 °C), and high temperature range (greater than 50 and less than or equal to 70 °C). The greatest dissimilarity was seen between the low temperature group and the high temperature group. A separation of sampling sites by pH could also be seen. These samples clustered into acidic or acidic to neutral pH groups, with the greatest dissimilarity being seen between the most acidic and acidic to neutral pH groups. However, the clustering was not as clear as the temperature clustering, indicating that temperature was a more important driver than pH (**Figure 2.3-11B**). Overall, this indicates that the microbial composition of fumaroles at Tramway Ridge respond to pH and temperature and highlights the gain in information obtained from increasing the dataset.

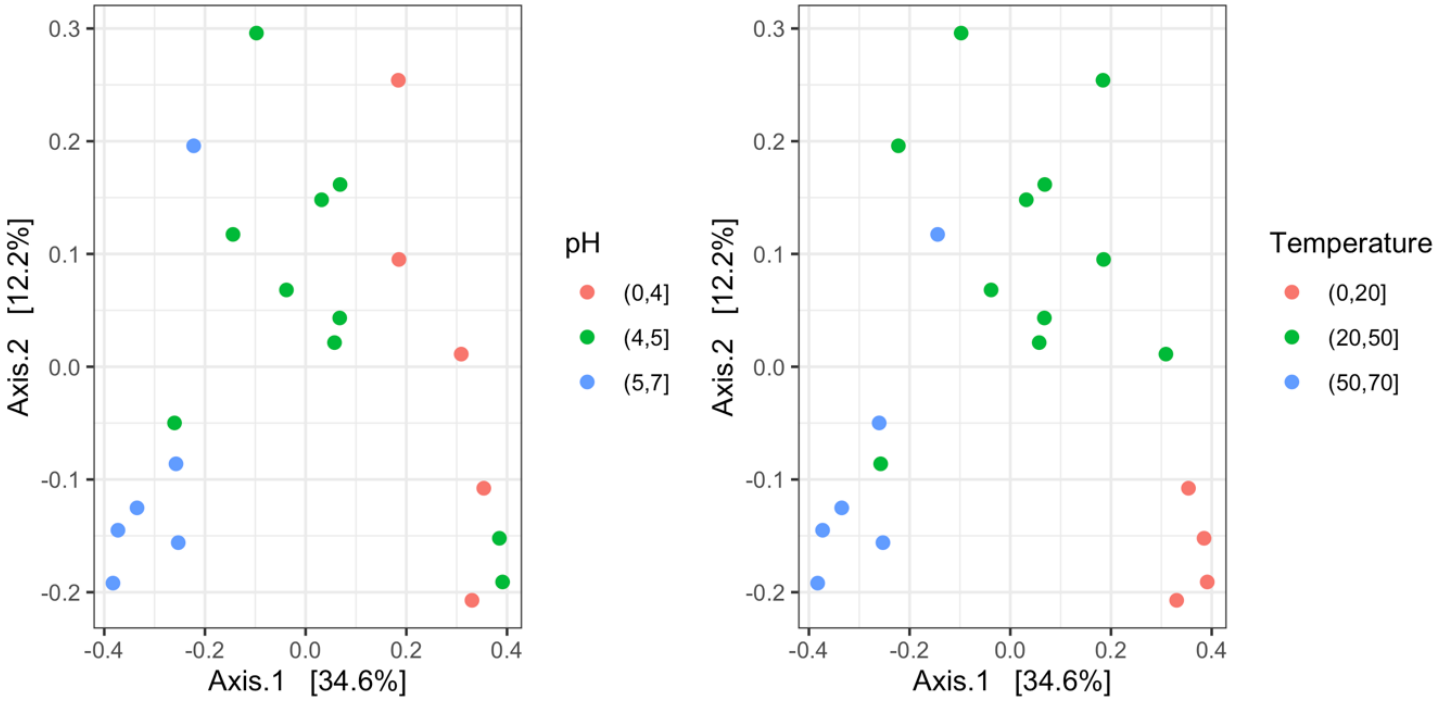


Figure 2.3-11: PCoA using weighted UniFrac of the sites examined on Mt. Erebus. A comparison of Tramway Ridge transects from this study (TR1 and TR2) and transect samples from previous years described in Herbold et al. (2014); Soo et al. (2009) grouped by A) pH and B) temperature. The total variance is explained by each axis in brackets

2.4 Discussion

Amplicon sequencing was used to assess the impact of temperature and geochemistry on soil microbial communities from two disparate geothermal sites on Mt. Erebus, Tramway Ridge and Western Crater, respectively. Three transects were analysed, two at Tramway Ridge and one at Western Crater, in order to compare microbial communities within site and between sites. As expected, the two transects within Tramway Ridge were more similar in their microbial communities than when compared to Western Crater. These differences in microbial communities' structure were considered to be mostly driven by pH since it varied more markedly between transects than any other physico-chemical parameters, enabling the separation of acidic and acidic to neutral soils at Tramway Ridge and neutral to alkaline soils at Western Crater. However, thermal and physico-chemical gradients, such as TC and TN content, and metal concentration, contributed to driving differences between different sampling sites within the same transect.

This study follows on from two previous studies performed at Tramway Ridge that looked at microbial communities in this geothermal site. Soo et al. (2009) examined temperature and physico-chemical gradients across three transects next to an active fumarole (strongly steaming) and used a culture-independent DNA barcoding (ARISA) approach to characterise microbial diversity. However, the resolution and throughput were much lower than the current genetic sequencing approaches of 16S rRNA gene amplicon analysis, which can differentiate amplicon sequence variants (ASVs). Herbold et al. (2014) examined a soil depth profile adjacent to an active and a passive 65 °C fumarole but did not undertake sequencing of the 16S rRNA gene amplicon for the microbial communities along a geochemical gradient. This study expands on the previous ones by examining thermal and geochemical gradients using high throughput sequencing of the 16S rRNA gene and discriminating those sequences as ASVs. Moreover, at Western Crater, the examination of microbial communities across a thermal and geochemical transect enabled the first characterisation of a Mt. Erebus geothermal site not impacted by active fumaroles.

2.4.1 Tramway Ridge

Tramway Ridge transects had comparable levels of TC, TN, EC, and GWC. The elevated total carbon and total nitrogen levels at Tramway Ridge are presumably due to the moss beds and cyanobacterial mats that could be seen on site adjacent to the fumaroles. Other studies have suggested that these mats provide the necessary nutrients for heterotrophic Archaea and Bacteria in other similar geothermal environments (Prieto-Barajas et al., 2018). Tramway Ridge, due to its terraces, is protected from the wind, which might allow the microbial mats to establish easier (Bolhuis et al., 2014) compared to other more exposed sites such as Western Crater.

The pH was strikingly different between both Tramway Ridge transects, with the TR1 sample sites being all acidic soils (ranging from 4–4.8) and the TR2 sample sites being composed of acidic to neutral soils (ranging from 4.5–6.2). Comparing these data with the two previous studies at Tramway Ridge, this was the first time that a completely acidic geothermal transect was analysed. Previous studies have reported transects with a pH variation similar to what was seen in this study for TR2 (Soo et al., 2009). However, the

impact of pH on the microbial community in TR1 and TR2 was not significant (*p*-value = 0.343) in that all sampling sites supported the same dominating phyla and the same dominating ASVs, at similar relative abundances.

Comparing the microbial communities' structure to previous characterisations of Tramway Ridge, this study showed that the phylum Crenarchaeota dominated all samples as reported by Soo et al. (2009) and Herbold et al. (2014). Specifically, Herbold et al. (2014) reported a single archaeal sequence accounted for 40–60 % of the amplicon libraries. This study also found one dominating archaeal sequence (ASV 1), comprising up to 45 % of the relative abundance at TR1 32 and an average relative abundance of 15 % across both Tramway Ridge transects. This ASV was the most abundant archaeal sequence seen in this study but differed from the most abundant sequence reported by Herbold et al. (2014). ASV 1 shared 86.4 % identity to *Nitrososphaera viennensis* on the NCBI 16S rRNA database (Appendix C). In contrast, the Deinococcota genus *Meiothermus*, reported in this study as ASV 5 and present at every Tramway sample site regardless of physico-chemistry, showed 100 % identity to (Appendix C) a sequence that had been reported before at 65 ºC by Herbold et al. (2014). The previous studies by Soo et al. (2009) and Herbold et al. (2014) also reported sequences assigned to the phylum Planctomycetes thriving at the hottest sampling sites. However, in this study, Planctomycetes could only be seen at two sampling sites, and neither were adjacent to the fumarole.

Proteobacteria could be seen in every sample of Tramway Ridge, which contrasted with the absence of this entire phylum reported by Soo et al. (2009). This was reported as a notable omission, given that Proteobacteria have been found to be dominant in other soil habitats in Antarctica (Aislabie et al., 2006; Ji et al., 2016; Niederberger et al., 2019). Interestingly, Herbold et al. (2014) reported a large number of sequences assigned to the phylum Proteobacteria, but only in two samples. This increased ability to detect Proteobacteria in the more recent studies at Tramway Ridge suggests that the enhanced resolution of 16S rRNA amplicon sequencing over the last few years has enabled a better characterisation of this major bacterial group. Primer bias has been reported before, specifically affecting Proteobacteria (Apprill et al., 2015; Parada et al., 2016). This work used primers with increased degeneracy, compared to Herbold et al. (2014), to increase

taxonomic coverage, which may partially explain the better detection of the Proteobacteria. Gammaproteobacteria was the most abundant Proteobacteria at Tramway Ridge, which might be attributed to the moist environment maintained by the condensing of steam from the active fumaroles. In the McMurdo Dry Valleys, Antarctica, Niederberger et al. (2019) and Tiao et al. (2012) showed that Gammaproteobacteria favoured soils with persistent moisture.

The concentration of Cu, Fe, Ni, S, and Zn was shown to vary between both Tramway Ridge transects. Metals may play a role in selecting archaeal and bacterial prevalence in a given environment (Sandaa et al., 1999). Previous studies have shown that in heavy metal contaminated soils, archaeal communities could increase in diversity at the same time that bacterial communities lost functionality of enzymes and the heavy metals interfered with nutrient cycling (Kandeler et al., 1996; Sandaa et al., 1999). However, in this work, the two sample sites that exhibited higher Archaea abundance (TR1 32 and TR2 44, with 45 % and 30 %, respectively) also had the highest and the lowest metal concentrations within their respective transects. This was interpreted as metal concentration probably not driving the high abundance of Archaea in any one sample. Alternatively, N could be playing a preponderant role. Both TR1 32 and TR2 44 exhibited the lowest N concentrations (< 0.15 %) seen in this study for Tramway Ridge. Archaea are known for being able to thrive in soils with low amounts of N, which contributes to a niche separation between Archaea and Bacteria (Di et al., 2010; Verhamme et al., 2011) and in this case, together with other physico-chemical factors, could be promoting the dominance of Archaea at these specific sampling sites.

The eight most abundant archaeal ASVs at Tramway Ridge (**Figure 2.3-6**) were all assigned to Class Nitrososphaerales, but taxonomic assignments at the lower ranks were different for different ASVs. Comparing this taxonomic assignment, obtained with the SILVA database, with the taxonomic assignment of the NCBI database, it could be seen that the latter showed all Archaea sequences affiliated to *Nitrososphaera viennensis* and sharing percentage identities between 86.40 and 99.19 %. This species is a mesophilic ammonia-oxidising archaeon that is a member of the Crenarchaeota (Thaumarchaeota in NCBI 16S rRNA database) phylum. It grows optimally at a neutral pH and 40 °C (Stieglmeier et al., 2014); the ASVs related to *Nitrososphaera viennensis* are mostly thermophilic, while two

are mesophilic. The higher nitrogen concentration at Tramway Ridge leads to increased nitrogen cycling and therefore increased ammonia fixing (You et al., 2009). This, in turn, supports these ammonia-oxidising taxa.

2.4.2 Western Crater

When compared to Tramway Ridge, Western Crater is a drier environment, probably due to the absence of active fumaroles and exposure to the high winds that are frequently recorded on Mt. Erebus (Bowman et al., 2015; Herbold et al., 2014), which may account for the lack of established moss beds and microbial mats. This may also account for the lower EC, GWC, TC and TN content seen at Western Crater, compared to Tramway Ridge.

Western Crater soils exhibited a more alkaline pH than Tramway Ridge that ranged from 6.9 to 9.2. The alkaline pH in the soils at Western Crater is more characteristic of the phonolitic nature of Mt. Erebus. (Kelly et al., 2008; LeMasurier & Thomson, 1990; Panter & Winter, 2008). Phonolite consists of alkali feldspar and nepheline (Kelly et al., 2008), contributing to the overall alkaline pH of the soil. The proximity to the lava lake and magma vent within the crater may cause increased exposure to the anorthoclase phonolite magma characteristic of Mt. Erebus (Kelly et al., 2008), further contributing to the elevated pH of the soil. The lack of moss might also be a consequence of the neutral to alkaline pH as mosses tend to thrive at a slightly acidic pH (Lee et al., 2010). On the other hand, cyanobacterial mats thrive under alkaline conditions (Roger, 1985), but since Western Crater sits at the top of the volcano, unprotected from the wind, a higher turnover of the top layers of soil and dispersive nature of cyanobacterial mats may account for the absence of established mats. In the previous studies at Tramway Ridge, Cyanobacteria has been linked to the occurrence of cyanobacterial mats (Herbold, Lee, et al., 2014; Soo et al., 2009). However, in the current study, a non-mat forming Cyanobacterium of the genus *Leptolyngbya* PCC-6306 was shown to be present at Western Crater (**Figure 2.3-6**). This was in addition to a low abundance Nostocaceae assigned to genus *Fischerella* PCC-9339 (ASV 36), that had 100 % identity with a sequence reported by Herbold et al. (2014) (Appendix C).

Six elements (Cu, Fe, Pb, S, V and Zn) exhibited similar concentrations across the WC transect except at WC 50, the sampling site with the highest temperature, which exhibited an increased concentration. These elements could be responding to the alkaline pH present at Western Crater. It has been reported that alkaline conditions extract copper and zinc from the soil, thus explaining their high concentration at the hotspot within the transect (Reed & Martens, 1996). At Tramway Ridge, it has been hypothesised that sulphur can contribute to a lower soil pH by converting to sulphuric acid in the presence of water, brought about by the gases released by the fumaroles (Soo et al., 2009). However, at Western Crater, the lack of active fumaroles means that sulphur is most likely not converting to sulphuric acid due to the lack of water; thus, a phonolite driven alkaline pH remains.

The Archaea present at Western Crater were in low diversity and abundance, in striking contrast to Tramway Ridge. At other Mt. Erebus sites, such as the three subglacial caves, no Archaea were found (Tebo et al., 2015). Surprisingly, Archaea have been conspicuously found in other Antarctica soils, specifically in the McMurdo Dry Valleys (Cary et al., 2010; Tebo et al., 2015) and Deception Island (Bendia et al., 2018).

The bacterial communities at Western Crater displayed a high relative abundance of Acidobacteriota, which are known to thrive at alkaline pH and low nutrient conditions (Ward et al., 2009). Another group which also are known to thrive in low nutrient environments are the Alphaproteobacteria (Cary et al., 2010), which were the most abundant Proteobacteria at Western Crater. Alphaproteobacteria have also been reported in Mt. Erebus subglacial caves (Tebo et al., 2015) and in cryptoendolithic environments at the McMurdo Dry Valleys (Cary et al., 2010). Planctomycetota were seen in every sampling site at Western Crater. Its presence has also been reported for other Antarctica environments such as subglacial caves on Mt. Erebus (Tebo et al., 2015), McMurdo Dry Valleys (Cary et al., 2010), and Tramway Ridge (Herbold, Lee, et al., 2014; Soo et al., 2009). Their presence across this broad range of terrestrial Antarctic habitats may be related to Planctomycetota's versatility and cosmopolitan character, found in many environments globally (Buckley et al., 2006).

This study obtained 16S rRNA amplicon sequencing data for Western Crater for the very first time. A striking number of ASVs (33 %) could not be assigned a taxonomic classification using the Bayesian classifier and the SILVA database. Thus, the high level of unclassified sequences could mean that the microbial communities present in Western Crater are highly unique and worthy of future study.

Interestingly, the genus *Meiothermus* reported in this study, which showed 100 % identity to a sequence that had been reported by Herbold et al. (2014), was absent from Western Crater, despite having been reported as a cosmopolitan organism (Herbold, Lee, et al., 2014). This suggests that either this organism is not cosmopolitan in Antarctica, it occupies specific niches where wind, moisture and nutrients are combined to ensure that viable airborne microbes can effectively colonise new habitats, or more likely, does not tolerate higher pH soils. Likewise, Crenarchaeota were thought to be prevalent on Mt. Erebus (Herbold et al., 2014), but the analysis of Western Crater showed that Tramway Ridge has physico-chemical characteristics that favour the narrower ecological niche that Archaea occupy, compared to Bacteria. These characteristics are most likely dictated by pH, nitrogen concentration and metal content.

2.4.3 Inter-Site Comparison

The most striking difference in the thermal and physico-chemical gradients at Tramway Ridge and Western Crater was pH. This could be explained by a heterogeneous magma source at Mt. Erebus, as hypothesised by Ilanko et al. (2019). The phonolitic nature of the lava lake in the main crater creates an alkaline environment at the summit and thus at Western Crater (R. P. Esser et al., 2004). The studies performed at Tramway Ridge so far are not conclusive regarding the most common soil pH. At the hottest sites, Soo et al. (2009) reported a pH of 7.4, Herbold et al. (2014) reported a pH of 8.63, whereas this study reported pH of 6.20 and 4.41, respectively. Herbold et al. (2014) argued that active and passive fumaroles had similar pH, but Ilanko et al. (2019) argued that there are multiple sources of emissions on Mt. Erebus, which may be leading to different geochemistry driving differing pH. Collectively, this might indicate that Tramway Ridge may actually be the exception to the rule and that the majority of the other sites around the volcano will be more like Western Crater. Further studies at Tramway Ridge will help us gain a better understanding of the heterogeneity within this particular geochemical site.

At Western Crater and Tramway Ridge, the microbial communities showed different richness and structure. The Shannon index showed lower richness of the microbial communities in the acidic soils of Tramway Ridge, with the richness of the microbial communities increasing as the pH of the soils increased at Western Crater. This is a trend that has previously been observed in elevated soils in the Tibetan plateau (Shen et al., 2019). Only 43 ASVs were common to all three transects, and a random forest analysis easily distinguished between the Tramway Ridge and Western Crater samples because of the high number of unique ASVs seen at Western Crater. Endemism has been suggested in the deeper soils at Tramway Ridge (Herbold, Lee, et al., 2014); therefore, these 43 shared ASVs could represent the endemic population of Mt. Erebus evolved to withstand the highly variable environmental conditions brought along by degassing events due to proximity to an active lava lake ((Tehnuka Ilanko, Oppenheimer, Burgisser, et al., 2015).

Although Tramway Ridge and Western Crater are only a kilometre apart, and the transects sampled at each site had a similar temperature range, but with differing physico-chemical characteristics, the microbial community composition and structure seen at each site were substantially different. This has been observed in previous geothermal studies that showed spatially close sites harboured unique microbial communities (Power et al., 2018; Takacs-Vesbach et al., 2008). When analysing the geothermal hot springs in Taupō Volcanic Zone (Power et al., 2018), saw that pH was a driver of microbial communities but only at temperatures above 70 °C, whereas other studies showed that pH was the strongest driver of bacterial diversity regardless of temperature (Bahram et al. 2018; Griffiths et al. 2011; Power et al. 2018; Shen et al. 2019). Overall, in this work, it has been seen that pH is the main driver that differentiates the microbial communities at two geothermal sites on Mt. Erebus.

2.4.4 Conclusion

This research has examined microbial communities from two disparate geothermal sites on Mt. Erebus. The study validated the assertion that variable and unique microbial communities are found on Mt. Erebus driven by physico-chemical site differences. The study showed that pH is a key driver of microbial community composition and structure through geochemical and statistical analysis. Collecting more data from Tramway Ridge has been invaluable to gain information to better characterise a valuable Antarctic Specially Protected Area (ASPA). This study has also shown that other geothermal sites on Mt. Erebus can be radically different from what was previously thought from the well-studied Tramway Ridge site widening our knowledge about the Mt. Erebus geothermal system. Future studies focussing on the functional analysis of these microbial communities will be invaluable to gain more insight into how the endemic taxa have adapted to the unique geothermal physico-chemistry of Mt. Erebus.

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Chapter 3
General Conclusion

This chapter is a brief conclusion and a look at further contributions to the research on Mt. Erebus, Antarctica.

The goal of this study was to determine the key physio-chemical drivers of microbial communities across three steep physico-chemical gradients at two disparate sites, Tramway Ridge and Western Crater. It was hypothesised that the unique physico-chemical features at each site would drive microbial community composition and abundance. The methodologies were used successfully to identify the key drivers of the microbial communities as well as to characterise the microbial communities. (See methods section in Chapter 2)

For the first time, Western Crater has been analysed using molecular and geochemical techniques, including the microbial communities, the physico-chemical properties of the soil, and how those physico-chemical properties drive the structure of the microbial communities. Additionally, all temperature samples of the transect at Tramway Ridge have been sequenced compared to previous studies that only sequenced the sample closest to the fumarole. This gives a full picture of the microbial community structure within the three transects across two geothermal sites, along with the associated geochemical properties of the samples.

IonTorrent sequencing provided quick and accurate sequencing to see the taxonomy present at each sample. Sequencing results were processed through the DADA2 pipeline to produce ASVs. This is the first time ASVs have been produced from soil samples taken from Mt. Erebus. This will allow the findings from this study to be compared to future studies to further our understanding of Mt. Erebus. dbRDA and PCoA analysis showed that pH is a critical factor in differentiating the samples from each site.

ICP-MS was used to measure the elements present in each sample. It showed that TR1 32 and WC 50 had an average of 38 % and 233 %, respectively higher than the next metal concentration of certain metals, leading to the conclusion that it allowed archaea to thrive. GWC and TC/TN were also used to analyse the physico-chemical drivers of the microbial communities on Tramway Ridge and Western Crater, Mt. Erebus, Antarctica.

Future characterisation of the volcano is important for the study of Mt. Erebus and Antarctica. This study will be able to be used as a comparison for future studies both on the surface of the volcano as well as any subsurface work.

Previous studies (Herbold, Lee, et al., 2014; Soo et al., 2009) have shown that microbial communities are linked to the subsurface biosphere. There has been limited subsurface research since 2009. Future studies should look at removing cores from these geothermal regions to examine if the hypothesised linked is an actual link between the surface communities and the subsurface communities.

The elemental analysis only determines the concentration of a small selection of common elements found in the earth's crust. A broader scope elemental analysis could give us a better understanding of Mt. Erebus due to its rare elemental composition (R. Esser et al., 2004). Previous studies have also conducted ICP-MS analysis which included elements not analysed in this study including: lithium, gallium, indium, and bismuth. In order to have a better comparison and understand how these sites change over time, future studies should include all elements that were previously studied in their future research because, in the last 14 years since Soo et al. (2009), these values have increased.

It has been shown recently that the full 16S rRNA sequence has higher resolution at the genus and species level and thus provides more information on the structure of the microbial communities. Moreover, at the species level, more information could be inferred regarding community functions. In the future, the DNA extracted in this study can be re-sequenced in order to obtain full 16S rRNA sequences.

Analysis of microbial eukaryotes has never been done in Tramway Ridge or Western Crater. It is known that microbial mats are found in Tramway Ridge but not in Western Crater, based on visual inspection when the samples were collected. A eukaryotic analysis of the geothermal sites would allow a better understanding of the entire ecosystem on Mt. Erebus.

Functional analysis has not been done at either site. This analysis would also provide information as to how these communities interact with their environment. A functional analysis on these samples could further explain their clustering patterns and the dissimilarities between the samples.

In order to determine the effects of the wind on the microbial communities and microbial mats between multiple locations on Mt. Erebus, the wind data must be collected at the sampling sites. This will allow future research to draw conclusions on the relationship between wind and microbial communities.

This study has determined the key physico-chemical drivers of microbial communities between active and passive fumarolic sites. For the first time, it has microbially and geochemically characterised Western Crater and sequenced microbial communities along the entirety of the geochemical gradient. This study has been the first step to a greater understanding of the unique and varied microbial communities across Mt. Erebus.

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Appendices

Appendix A **Physico-chemical**

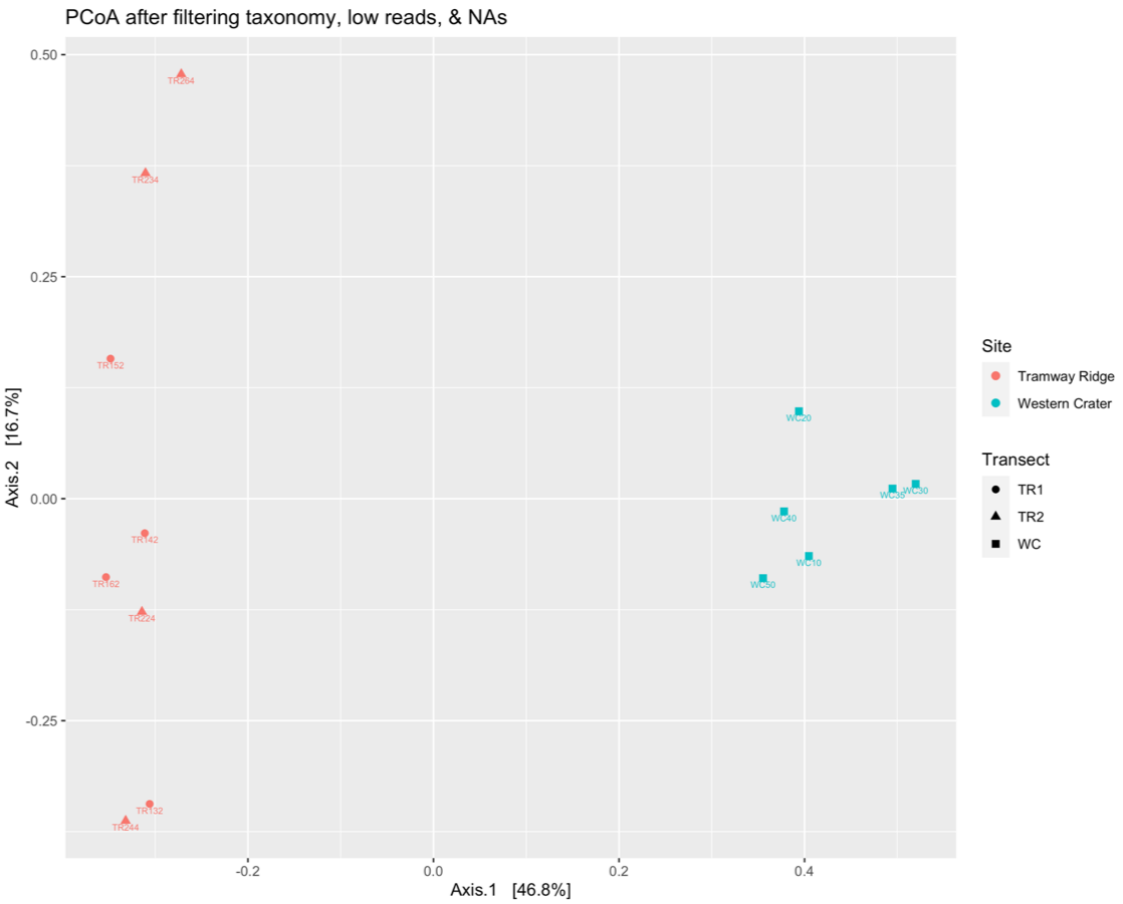
Appendix Table A-1: All 33 elements that were examined via ICP-MS

Sample Name	11 B	23 Na	24 Mg	27 Al	39 K	44 Ca	31-47 P	34-50 S	51 V	52 Cr	55 Mn	56 Fe	59 Co
TR132	81.665	9613.008	3176.773	46659.14	6519.03	4375.758	1815.532	956.104	2.724	44.199	1663.876	39936.289	1.94
TR142	78.143	4047.35	2243.022	142524.348	2692.619	5053.736	1552.572	597.407	1.73	17.223	1366.467	10924.022	1.058
TRI52	81.826	8281.324	2722.362	112740.323	5979.546	5109.474	1513.046	865.781	2.413	39.113	2177.696	17127.757	1.428
TRI62	76.503	3338.095	2258.68	117670.088	2435.7	2557.92	1730.17	968.652	1.294	50.252	1809.18	24367.393	1.243
TR224	79.89	2693.617	2299.258	39088.427	2109.439	1568.983	873.164	1598.221	7.285	23.373	2540.601	61122.351	2.402
TR234	84.522	12882.745	2435.435	93913.38	9131.028	3322.22	1227.463	799.177	4.738	50.47	2024.355	57661.994	2.044
TR244	82.945	8422.667	5531.541	84470.269	6283.748	7687.348	2538.689	264.398	0.759	41.99	1174.66	17816.478	1.489
TR264	82.114	21195.863	4201.867	76003.437	14834.971	6711.516	1283.913	866.023	8.546	58.341	1732.134	69908.916	2.585
WC10	80.877	10365.337	5277.528	79692.294	7864.462	7752.413	2646.477	245.425	0.836	59.748	1106.674	20184.889	1.855
WC20	79.717	10570.557	7565.6	87388.592	8032.508	8597.018	2783.775	219.324	2.455	87.779	1369.972	21114.197	2.174
WC30	81.269	11190.426	5214.091	59021.521	7430.697	7018.928	2270.54	248.89	<0.000	75.629	1041.135	17488.914	1.625
WC35	81.408	10993.123	5445.726	83805.662	8383.897	7907.702	2252.156	290.487	<0.000	64.816	1373.883	17977.07	1.486
WC40	78.971	7357.252	6449.164	73970.092	5703.581	8036.959	2535.137	137.89	1.246	57.738	1207.972	19034.804	1.618
WC50	81.289	14740.547	3482.424	79960.45	10330.685	4535.759	1249.752	1781.111	6.659	29.254	1816.339	75346.108	2.44

Sample Name	60 Ni	65 Cu corrected	66 Zn	68 Zn	75 As	78 Se	82 Se corrected	88 Sr	75 -> 91 As [N2O + H2]	78 -> 94 Se [N2O + H2]
TR132	2.844	1.781	74.481	79.655	8.309	2.995	5.03	50.597	6.24	<0.000
TR142	0.478	0.487	36.329	49.149	16.553	13.879	25.213	27.305	3.64	0.015
TRI52	0.552	0.941	54.026	69.679	14.091	11.225	20.369	32.352	4.05	<0.000
TRI62	0.655	1.093	55.024	66.828	11.455	10.566	19.94	23.825	1.41	<0.000
TR224	0.96	1.646	85.937	90.237	5.35	2.393	4.832	44.925	4.236	<0.000
TR234	1.626	2.259	113.999	114.749	5.071	2.45	4.508	62.984	3.139	<0.000
TR244	0.75	0.916	48.533	58.073	9.674	8.152	14.874	50.286	2.122	<0.000
TR264	3	2.255	121.961	123.976	11.163	3.838	6.199	99.537	9.132	<0.000
WC10	1.802	1.111	58.086	63.533	8.351	6.824	11.625	47.927	1.793	<0.000
WC20	1.417	1.296	65.978	72.283	8.565	7.336	12.512	52.86	1.72	<0.000
WC30	1.16	1.131	52.473	57.924	6.944	5.893	10.141	47.565	1.599	<0.000
WC35	1.596	0.986	46.432	55.681	10.044	8.188	15.171	52.737	1.926	<0.000
WC40	0.928	0.809	45.918	54.086	10.983	9.128	15.803	59.711	2.661	<0.000
WC50	1.707	3.087	121.98	121.815	7.416	2.774	4.497	68.456	5.481	<0.000

Sample Name	82 -> 98 Se [N2O + H2]	107 Ag	111 Cd	137 Ba	201 Hg	205 Tl	206 Pb	207 Pb	208 Pb	238 U
TR132	<0.000	<0.000	0.771	298.781	<0.000	0.106	30.97	27.691	30.023	5.152
TR142	<0.000	<0.000	4.529	1161.169	<0.000	0.238	26.884	23.971	26.1	14.628
TRI52	<0.000	<0.000	5.17	1237.094	<0.000	0.273	32.603	29.116	31.642	11.502
TRI62	<0.000	<0.000	2.501	943.805	<0.000	0.17	33.273	29.736	32.49	12.119
TR224	<0.000	<0.000	0.948	295.181	<0.000	0.169	15.403	13.697	14.385	5.485
TR234	<0.000	<0.000	4.22	283.63	<0.000	0.202	15.377	13.743	14.431	6.611
TR244	<0.000	<0.000	0.423	637.617	<0.000	0.091	11.55	10.344	10.855	11.735
TR264	<0.000	<0.000	2.544	374.685	<0.000	0.123	55.925	49.926	54.064	8.782
WC10	<0.000	<0.000	0.264	388.232	<0.000	0.045	7.335	6.582	6.889	9.127
WC20	<0.000	<0.000	0.382	507.75	<0.000	0.065	8.898	7.977	8.356	10.216
WC30	<0.000	<0.000	0.326	353.47	<0.000	0.074	7.46	6.668	6.999	8.062
WC35	<0.000	<0.000	0.467	640.351	<0.000	0.099	12.116	10.791	11.342	11.616
WC40	<0.000	<0.000	0.419	589.806	<0.000	0.072	10.677	9.579	10.024	11.193
WC50	<0.000	<0.000	0.716	257.915	<0.000	0.08	40.238	35.97	39.11	7.464

Appendix B **Sequencing Quality Control**



Appendix Figure B-1: PCoA of the relative abundance after erroneous taxonomy, low read samples, and unclassified ASVs were removed.

Appendix C Taxonomy of ASVs

Appendix Table C-1: ASVs Found in Previous Studies

ASV	SILVA	NCBI 16S rRNA Database	Percent Similarity	Accession Number	Nucleotide Collection	Percent Similarity	Accession Number	Previous Study
ASV_5	Bacteria, Deinococcota, Deinococci, Thermales, Thermaceae, Meiothermus	Bacteria, Deinococcus-Thermus, Deinococci, Thermales, Thermaceae, Meiothermus, granaticius	93.9	NR_117536.1	uncultured bacteria	100	KF923322.1	HERBOLD
ASV_24	Bacteria, Deinococcota, Deinococci, Thermales, Thermaceae, Meiothermus	Bacteria, Deinococcus-Thermus, Deinococci, Thermales, Thermaceae, Meiothermus, silvanus	95.53	NR_074273.1	Uncultured bacterium clone ERBTW20082009	100	KF923322.1	HERBOLD
ASV_36	Bacteria, Cyanobacteria, Cyanobacteriia, Cyanobacteriales, Nostocaceae, Fischerella PCC-9339	Bacteria, Cyanobacteria, Nostocales, Hapalosiphonaceae, Fischerella, muscicola	99.19	NR_112177.1	uncultured bacteria	100	KF923320.1	HERBOLD
ASV_61	Bacteria, Acidobacteriota, Blastocatellia, Pyrinomonadales, Pyrinomonadaceae, Pyrinomonas	Bacteria, Acidobacteria, Blastocatellia, Blastocatellales, Pyrinomonadaceae, Pyrinomonas, methylaliphatogenes	99.19	NR_133835.1	Uncultured bacterium clone ERBTW20082009OTU078	100	KF923318.1	HERBOLD
ASV_47	Bacteria, Chloroflexi, Anaerolineae, SBR1031, NA, NA	Bacteria, Firmicutes, Bacilli, Bacillales, Thermoactinomycetaceae, Polycladomyces, subterraneus	83.47	NR_158012.1	uncultured bacteria	100	EU490269.1	SOO

Appendix Table C-2: Key ASVs Identified by Random Forest Classified by SILVA and NCBI

ASV	SILVA	NCBI 16S rRNA Database	Percent Similarity	Accession Number	Nucleotide Collection	Percent Similarity	Accession Number
ASV_2	Bacteria, Proteobacteria, Gammaproteobacteria, Xanthomonadales, Rhodanobacteraceae, Mizugakiibacter	Bacteria, Proteobacteria, Gammaproteobacteria, Xanthomonadales, Rhodanobacteraceae, Mizugakiibacter, Sediminis	95.12	NR_126196.1	uncultured bacteria	99.19	MH760844.1
ASV_10	Archaea, Crenarchaeota, Nitrososphaeria, Nitrososphaerales, Nitrososphaeraceae, Candidatus Nitrososphaera	Archaea, Thaumarchaeota, Nitrososphaeria, Nitrososphaerales, Nitrososphaeraceae, Nitrososphaera, Viennensis	97.15	NR_134097	Uncultured bacterium clone VG_QRY_9	100	KM278315.1
ASV_31	Bacteria, Proteobacteria, Gammaproteobacteria, Gammaproteobacteria Incertae Sedis, Unknown Family, Acidibacter	Bacteria, Proteobacteria, Gammaproteobacteria, Gammaproteobacteria incertae sedis, Acidibacter, ferrireducens	98.37	NR_126260.1	Uncultured bacterium clone	99	MH977817.1
ASV_122	Bacteria, Actinobacteriota, Acidimicrobiia, NA, NA, NA	Bacteria, Actinobacteria, Acidimicrobiia, acidobacteriales, acidimicrobiaceae, aciditerrimonas, ferrireducens	95.53	NR_112972.1	Uncultured bacterium	98.37	MT605230.1
ASV_134	Bacteria, Planctomycetota, Planctomycetes, Gemmatales , Gemmataceae, NA, NA	Bacteria, Planctomycetes, Planctomycetia, Gemmatales, Gemmataceae, Limnoglobus, roseus	87.15	NR_169492	Uncultured bacterium clone ERB-D10 16S ribosomal RNA gene, partial sequence	100	EU490275.1
ASV_16	Bacteria, GAL15, NA, NA, NA, NA	Bacteria, Firmicutes, Clostridia, Thermoanaerobacterales, Thermoanaerobacteraceae, brockia, lithotrophica	89.88	NR_132331.1	Uncultured Firmicutes bacterium clone GASP-45KA-41-A06	99	EU044230.1

ASV_25	Bacteria, Proteobacteria, Gammaproteobacteria, Gammaproteobacteria Incertae Sedis, Unknown Family, Acidibacter	Bacteria, Proteobacteria, Gammaproteobacteria, Gammaproteobacteria incertae sedis, Acidibacter, ferrireducens	96.34	NR_126260.1	uncultured bacteria/prokaryote	99.59	C302594.1
ASV_33	Bacteria, Planctomycetota, Planctomycetes, Gemmatales, Gemmataceae, NA	Bacteria, Planctomycetes, planctomycetia, gemmatales, Gemmataceae, gemmata, massiliana	87.85	NR_148576.1	Uncultured bacterium	100	MH528489.1
ASV_35	Bacteria, Acidobacteriota, FFCH5909, NA, NA, NA	Bacteria, Proteobacteria, Alphaproteobacteria, Rhodospirillales, Rhodospirillaceae Azospirillum, canadense	88.84	NR_117877.1	uncultured bacteria	95.12	KR840277.1
ASV_43	Bacteria, Cyanobacteria, Cyanobacteriia, Cyanobacteriales, Nostocaceae, Mastigocladopsis PCC-10914	Bacteria, uncultered bacteria	100	NR_074317	uncultured bacteria	100	MF968396.1
ASV_57	Bacteria, Chloroflexi, AD3 , NA, NA, NA	Bacteria, Firmicutes, bacilli, bacillales, Bacillaceae, Caldalkalibacillus, thermarum	88.71	NR_043169.1	Uncultured bacterium clone OTU_28	99.59	MK724095.1
ASV_63	Bacteria, Chloroflexi, Ktedonobacteria, Ktedonobacterales, Ktedonobacteraceae, G12-WMSP1	Bacteria, Chloroflexi, ktedonobacteria, Thermogemmatisporales , Thermogemmatisporaceae, Thermogemmatispora, carboxidivorans	85.48	NR_133881.1	Uncultured prokaryote gene for urease	94	LC305072.01
ASV_68	Bacteria, Planctomycetota, Planctomycetes, Gemmatales, Gemmataceae, NA	Bacteria,Actinobacteria, Acidimicrobiia, Acidimicrobiales, Acidimicrobiaceae, Aciditerrimonas, ferrireducens	92.28	NR_112972.1	Uncultured bacterium clone 4738	100	KU542368.1

ASV_71	Bacteria, Acidobacteriota, Holophagae, Subgroup 7 , NA, NA, NA	Bacteria, Proteobacteria, Alphaproteobacteria, Rhodospirillales, Rhodospirillaceae, Thalassobaculum, salexigens	85.89	NR_149296.1	Uncultured bacterium clone GP27677fO8 16S ribosomal RNA gene, partial sequence	98.78	HM445435
ASV_82	Bacteria, Planctomycetota, Planctomycetes, Gemmatales , Gemmataceae, NA	Bacteria, Planctomycetes, planctomycetia, Gemmatales, Gemmataceae, Limnoglobus, roseus	86.75	NR_169492.1	Uncultured bacterium clone OTU_19410	100	KR850694.1
ASV_93	Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Pedomicrobium	Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae, Pedomicrobium, australicum	97.56	NR_104908.1	uncultured bacterium	100	LR589858.1
ASV_116	Bacteria, Acidobacteriota, Holophagae, Subgroup 7, NA, NA, NA	Bacteria, Proteobacteria, Alphaproteobacteria, Hyphomicrobiales, Hyphomicrobiaceae, Methyloligella, solikamskensis	87.8	NR_125614.1	Uncultured bacterium partial 16S rRNA gene, Mn sample, clone 60	99.59	HG003474.1
ASV_145	Bacteria, Methylomirabilota, Methylomirabilia, Rokubacteriales, NA, NA	Bacteria, Firmicutes Clostridia, Eubacteriales, Eubacteriales incertae sedis, Clostridiales Family XVII. Incertae Sedis, Thermaerobacter	83.61	NR_074944.1	Uncultured bacterium clone A1767 16S ribosomal RNA gene, partial sequence	100	MF048349.1
ASV_171	Bacteria, Proteobacteria, Alphaproteobacteria, Rickettsiales, Mitochondria, NA	Bacteria, Bacteroidetes, Cytophagia, Cytophagales, Flammeovirgaceae, Perexilibacter, aurantiacus	72.64	NR_041534	Pectinodesmus pectinatus mitochondrion, complete genome	83.4	KT946995.1